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TITLE OF THESIS PROTEIN INCORPORATION IN RAT HEART MUSCLE FOLLOWING ACUTE SPRINT OR ENDURANCE RUNNING

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THE UNIVERSITY OF ALBERTA

# PROTEIN INCORPORATION IN RAT HEART MUSCLE FOLLOWING ACUTE SPRINT OR ENDURANCE RUNNING

by

DAVID WILLIAM WILES

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled PROTEIN INCORPORATION IN RAT HEART MUSCLE FOLLOWING ACUTE SPRINT OR ENDURANCE RUNNING submitted by DAVID WILLIAM WILES in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSPHY.

# DEDICATION

To Wendy, whose love, faith and motivation were the driving forces that resulted in the completion of this degree.

ABSTRACT

This study was designed to gain some insight into how acute exercise, of differing intensities, effect protein metabolism in the heart. The design allowed the chronic, acute and post exercise response patterns to be examined. Following the 2½ week acclimation programs the animals were required to perform their persective anaerobic or aerobic performance protocols. This involved sprinting at 90m/min for 10 seconds, followed by a 20 second rest up a 10% grade repeated 60 times, for the anerobic groups. The aerobic animals were required to run continuously at 30m/min up a 10% grade for 30 minutes. The effects of the forementioned acclimation and performance protocols on protein metabolism, was inferred by the incorporation of the radioactively labelled amino acid leucine, into the myofibrillar-nuclear, mitochondrial and soluble fractions of heart muscle. The in vivo incorporation of leucine into protein into the different heart fractions was expressed as specific activity in decays/min/mg of protein. Animals were sacrificed and their hearts removed for the subsequent biochemical analysis either pre-exercise or at 0, 12, 24, 36, 48, 60 or 72 hours post exericse. Due to the limited sample size, (3 animals per group) and the heterogeneity in the response patterns, the following discussion is based on the trends that were evident in the data. Both the anaerobic and aerobic acclimation programs resulted

in a reduction in the rate of amino acid incorporation. This supports the hypothesis that exercise has a greater effect on protein degradation than on synthesis. The acute effect of exercise on protein metabolism appears dependent upon the nature of the exercise and the muscle fraction examined. Following anaerobic exercise the incorporation rate increased in the myofibrillar-nuclear fraction while it decreased in the mitochondrial and soluble fractions. Following aerobic exercise the activity increased in all three fractions. Finally anaerobic and aerobic exercise appear to differentially effect the post exercise response patterns for protein uptake (ie. the response is both more pronounced and delayed following anaerobic exercise as compared to aerobic exercise. It is suggested that the variability in the amino acid uptake evident within the experimental groups, may be indicative of differences in the relative intensity of the imposed exercise stimuli on the individual animals.

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# I INTRODUCTION

Nearly a century ago, exercise physiologists began studies of muscular adaptations induced by physical activity (Keys, 1971). With the advent of modern biochemical procedures, research has progressed from descriptive analysis to quantitative physiological and biochemical studies. Results from such investigations have been beneficial toward gaining a more complete understanding of the effects of exercise at the cellular level. Research dealing with heart muscle is concerned not only with its role in blood circulation and the subsequent effect of an athletic performance, but also with the influence of exercise on the prevention and rehabilitation of heart disease.

Environmental factors such as a chronic exercise stimulus are capable of inducing marked adaptations in skeletal muscle. Resulting adaptations whether they are structural or functional are specific to the nature of the exercise stress incurred. That is, endurance training results in an enhanced capacity for energy production via oxidative phosphorylation, (Baldwin et al., 1972; Jaweed et al., 1974; Holloszy, 1975), while sprint training brings about changes in the capacity for glycolytic metabolism, (Baldwin et al., 1977; Sjodin et al., 1976) and the contractile functions, (Gordon et al., 1967; Jaweed et al., 1974). The responses of skeletal muscles to exercise have been demonstrated in both animal and human subjects (Thorstensson et al., 1975).

Research on the response of heart muscle to exercise has not produced as lucid an understanding as those studies dealing with skeletal muscle. Due to the inaccessibility of the heart for biochemical studies in man the majority of research has been undertaken using animal models. Inherent to these models, there are several experimental design problems which contribute to the current controversy over the adaptability of the heart to exercise training (Segel, 1977). The major problems can be summarized as follows; age of the animal at the beginning and end of the conditioning program, type of exercise and the intensity of the training program, type of anethesia used before sacrifice, diet and the influence of the final exercise bout on the results. Also it is desirable to determine, in addition to measuring quantitative changes in constituent proteins of heart, whether or not another criterion of exercise effect such as an increase in the oxidative energy production is effected.

Despite these confounding factors, some generalizations can be made. Heart muscle has been shown to hypertrophy in response to an exercise program (Baldwin et al., 1977; Dowell et al., 1976; Medugorac, 1976). The benefits of this hypertrophy are demonstrated by the ability of the organ to maintain its function under stress conditions (Schaible et al., 1979; Dowell et al., 1976; Carey et al., 1976). It appears that at the tissue level, there are only minimal changes in the hearts metabolic function. The activity of

respiratory enzymes and mitochondrial protein concentration in response to exercise are elevated in concert with the increase in cardiac size (Oscai et al., 1971; Dohm et al., 1977; Baldwin et al., 1975). It is apparent when comparing trained and untrained hearts that exercise does not cause any relative difference in biochemical parameters. It may be that the most critical factor governing training adaptations of cardiac tissue is the relative intensity of the exercise stress imposed on animals (Baldwin et al., 1977; Bostrom et al., 1974). The failure to demonstrate relative changes in the biochemical parameters of the heart, may be related to either insufficient overload being placed on the heart by the training program, or the inability of the animals to perform at the intensity level required to produce changes.

Regardless of the nature of the physiological adaptations induced by chronic exercise, they are a consequence of an alteration in the protein composition of the muscle. The majority of studies to date have been concerned with the final result of a training program. This final result or training adaptation, is the cummulative effect of the acute exercise bouts. Few studies have focused on how an acute exercise bout effects the cellular components involved in the regulation of protein metabolism. If in fact the intensity of a training stimulus is considered a key factor in governing the nature of a training adaptation, it is hypothesized that the resultant effects of acute anaerobic and aerobic exercise on leucine incoporation would be different.

This study was designed to gain some insight into how acute bouts of different intensities of exercise effect protein metabolism in the heart. The study uses five treatment programs, a control, sprint acclimation training, a sprint exercise bout, endurance acclimation training and an endurance exercise bout. These programs enable the evaluation of the effects of two different acclimation programs (sprint and endurance), and two different intensities of an exercise stimuli (sprint and endurance running) on protein metabolism. The effects of the sprint and endurance exercise bouts are examined immediately post exercise and at twelve hour intervals, up to seventy-two hours. In order to measure the effects of the above programs on protein metabolism in the heart, three dependent variables are utilized. These variables are the myofibrillar-nuclear (myo), mitochondrial (mito) and soluble (sol) fractions of heart muscle. Protein metabolism is inferred by the incorporation of radioactivity labelled leucine into the different fractions. These fractions were selected for they will be representative of changes in the hearts contractile protein (myo), and in the capacity of the heart for aerobic (mito) and anaerobic (sol) energy production. The specific purposes of this study are:

I To examine whether an acclimation exercise program of either anaerobic (sprint or aerobic (endurance) training, differentially effects protein metabolism in separate fractions of heart muscle.

II To determine the immediate effects of an acute sprint or endurance exercise stimulus on protein metabolism in different fractions of heart muscle.

III To examine the response in protein metabolism in different heart fractions during the time period from 12 to 72 hours following exposure to either a sprint or endurance exercise stimulus.

Due to the exploratory nature of the study, (the effects of exercise on protein metabolism being examined over seven separate time periods), and the time and cost involved in tissue analysis, only three animals per group were used. Due to this small number the results can only be considered as tentative and providing directions for further investigation.

## II METHODOLOGY

### A ANIMAL CARE

Seventy male Wistar rats, specific pathogen free, were obtained from the Charles River farms in Newfield, N.J.. The rats were approximately five weeks of age ranging in weight from 100 to 125 grams at the onset of the experimental period. Following their arrival at the laboratory the animals were subjected to a six day laboratory orientation period. All animals were housed in pairs in wire mesh cages in a temperature controlled room maintained at 22°C. Due to the nocturnal nature of rats, the day/night cycle was reversed to facilitate their training, (twelve hour rotation, night cycle from 0700-1900 hours). The rats were fed and watered ad libitum on a diet of Purina Rat Chow, Ralston Purina Ltd., St. Louis, Missouri (23% crude protein). The daily routine consisted of handling of all animals, rotation of cages on the rack to reduce any effects of stratification of temperature and air supply, changing soiled papers and replenishment of food and water. The cages and water bottles were washed and sterilized. The rats were weighed weekly and weights were recorded in grams, Table VIII. All environmental factors were controlled within the guidelines as set down by the Canadian Council of Animal Care to ensure a constant response to the experimental program. At the conclusion of the six day orientation period all animals were assessed to ascertain their treadmill running ability. This was done by a subjective evaluation of the animals willingness to run. The evaluation was made from

two separate sessions on the treadmill. The first exposure was approximately 30 seconds in duration at 10 m/min. and 0% grade, the second exposure was approximately 1 min. in duration at 15 m/min. and 0% grade. Fifty four of the seventy animals obtained were judged suitable to perform the running task. These animals were then randomly assigned to either a control, sprint or endurance group.

## B PERFORMANCE ACCLIMATION PROTOCOL

All acclimation exercise sessions were performed on a motor driven Quinton rodent treadmill (Model 2A) which has ten compartments (9.5cm x 48cm). Each compartment contains an electrical grid, a device initially used to provide an electric shock to motivate the animals to run. At the start of the acclimation program the acclimated and performance animals were conditioned to associate the electric shock with a squirt of water. This Pavlovian response was sufficiently developed by the end of the first week of acclimation, that when necessary, only the water was required to reestablish an animals running pattern. Exercise sessions were conducted twice daily (at approximately 0900 hours and 1400 hours) four days per/week (i.e., Mon., Tues., Thurs., Fri.).

The respective training programs for the aerobic (endurance) and anaerobic (sprint) groups are outlined in Tables I and II. They were designed to progressively increase the load on the animals so that upon completion of the program they were able to complete their particular

performance protocols.

C PERFORMANCE PROTOCOL

Following the two and one half week acclimation program the aerobic and anaerobic animals were given a seventy two hour rest period prior to the performance of the final exercise task. The performance protocol for the anaerobic group consisted of a single bout of intermittent training on the treadmill at a speed of 90m/min. and 10% grade. During the exercise period, cycles of work (10 sec.) and recovery (20 sec.) were repeated 60 times. The aerobic group were trained to perform a single bout of continuous training, running on the treadmill at a speed of 30m/min. at 10% grade for a duration of 30 minutes. With this format the total work performed by both groups was equal.

Table I: Details the method of overload utilized in the performance acclimation program for the anaerobic (sprint) group in preparation for them to complete the performance task.

| Day (No) | Time | Speed (m/min) | Grade (%) | Duration (10:20s) |
|---|---|---|---|---|
| 1 | am | 10 | 0 | 3min |
| 1 | pm | 15 | 0 | 3min continuous |
| 2 | am | 20 | 5 | 3min running |
| 2 | pm | 20 | 5 | 3min |
| 3 | am | 20 | 5 | 6 repetitons |
| 3 | pm | 20 | 5 | 6 |
| 4 | am | 40 | 5 | 8 |
| 4 | pm | 50 | 5 | 8 |
| 5 | am | 50 | 10 | 10 |
| 5 | pm | 50 | 10 | 12 |
| 6 | am | 60 | 10 | 12 |
| 6 | pm | 60 | 10 | 12 |
| 7 | am | 70 | 10 | 16 |
| 7 | pm | 70 | 10 | 16 |
| 8 | am | 80 | 10 | 16 |
| 8 | pm | 80 | 10 | 20 |
| 9 | am | 90 | 10 | 20 |
| 9 | pm | 90 | 10 | 20 |
| 10 | am | 90 | 10 | 24 |
| 10 | pm | 90 | 10 | 24 |

TABLE II: Details the method of overload utilized in the performance acclimation program for the aerobic (endurance) group in preparation for them to complete the performance task.

| Day (No) | Time | Speed (m/min) | Grade (%) | Duration (min) |
|---|---|---|---|---|
| 1 | am | 10 | 0 | 3 |
| 1 | pm | 15 | 0 | 3 |
| 2 | am | 20 | 5 | 3 |
| 2 | pm | 20 | 5 | 3 |
| 3 | am | 20 | 5 | 4 |
| 3 | pm | 20 | 5 | 4 |
| 4 | am | 30 | 5 | 5 |
| 4 | pm | 30 | 5 | 5 |
| 5 | am | 30 | 10 | 6 |
| 5 | pm | 30 | 10 | 6 |
| 6 | am | 30 | 10 | 7 |
| 6 | pm | 30 | 10 | 7 |
| 7 | am | 30 | 10 | 8 |
| 7 | pm | 30 | 10 | 8 |
| 8 | am | 30 | 10 | 9 |
| 8 | pm | 30 | 10 | 9 |
| 9 | am | 30 | 10 | 10 |
| 9 | pm | 30 | 10 | 10 |
| 10 | am | 30 | 10 | 12 |
| 10 | pm | 30 | 10 | 12 |

D TISSUE HANDLING

At designated time intervals (Table III) following completion of the performance protocol the following procedures were followed. Ten minutes prior to sacrifice the animal was restrained and injected via the saphenous vein with L-(4, $5^{3}$H) -Leucine (25 MCi per 100 gm body weight) in a physiological saline solution. After the 10 minute incubation period the animal was sacrificed by decapitation. A whole blood sample was collected (approximately 5 ml) and used to determine circulating levels of radionucleide. The chest cavity was opened, the heart isolated, removed and washed in an ice cold physiological saline solution. The heart was then trimmed, blotted and weighed. A sample (approximately 150 mg) of myocardium was taken from the apex of the organ for subsequent biochemical analysis. From the time of injection to the time of tissue processing required approximately fifteen minutes. The rational for the selection of leucine as the marker for amino acid incorporation was as follows. In the albino rat leucine is an essential amino acid, as such it is not synthesized and must be obtained from an exogenous source, Lehninger 1970. Leucine is metabollically stable in that it is not readily converted to another amino acid, Zamecnik 1969. Morgan et al (1971) demonstrated that leucine readily equilibrates between the extra and intracellular spaces in heart muscle. Finally, the concentration of leucine in the plasma and muscle has been shown not to be altered by physical training, Dohm 1977.

TABLE III: Anaerobic or sprint (AN.) and aerobic or endurance (AE.) experimental groups and post exercise sacrifice schedule chosen for observation. (SED.C.-sedentary control; ACC.C.-acclimated control).

| EXERCISE TREATMENT | EXPERIMENTAL GROUPS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Sacrifice Times (HRS.) | | | | | | |
| | SED.C. | ACC.C. | 0 | 12 | 24 | 36 | 48 | 60 | 72 |
| AN. | 3* | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| AE. | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

* Number of Animals per Cell

E BIOCHEMICAL PROCEDURES

The sample of heart tissue was placed in a chilled homogenizing solution containing 0.32 M sucrose, 10.0 mM cold leucine and 5.0 mM EDTA (ph 7.4) to a volume of 3.0 ml/100 ml wet weight muscle. The muscle was then minced with chilled scissors and homogenized with a Polytron homogenizer (Brinkman Instr.) by a single 5 second burst, at a rehostat setting of 5.

TOTAL HOMOGENATE

An aliquot (0.5 ml) was taken from the above suspension. To this 0.1 ml of 10% SDS was added and left to dissolve for 10 minutes at room temperature. The proteins contained in the homogenate were precipitated by the addition of 3.0 ml 20% TCA and isolated by centrifugation (1000 x g, 10 minutes). The resulting pellet was washed and re-centrifuged three times with 5.0 ml of 10% TCA wash solution containing 10 mM leucine. Following the final centrifugation the pellet obtained was dissolved in 1.0 ml 0.5 N NaOH at $36^{0}$C. Aliquots were taken in duplicate for the determination of protein content and the radioactive counting.

MYOFIBRILLAR-NUCLEAR FRACTION

The remaining suspension from the initial homogenation procedure was centrifuged at 2500 x g for 10 minutes 2-$4^{0}$C (Ivan Sorval model model RC2B, HB4 rotor). The supernatant was decanted, collected and utilized for the mitochondrial and soluble fraction while the pellet was used for the myofibrillar-nuclear fraction. The pellet was resuspended

in 3 ml of the original homogenization buffer and centrifuged at 2500 x g for 10 minutes. The supernatant was decanted and discarded. The remaining pellet was then washed and centrifuged twice with the same volume of homogenization solution. Following the final centrifugation, the remaining pellet was dissolved in 1.0 ml of 0.5 N NaOH at $36^{0}$C. Aliquots were taken in duplicate for protein determination and the radioactive counting.

MITOCHONDRIAL FRACTION

The supernatant from the first centrifugation of the myofibrillar-nuclear preparation was transfered into 15 ml Corex tubes and centrifuged at 12000 x g for 15 minutes. The supernatant from this spin was collected and utilized for isolation of the soluble fraction. The remaining pellet was washed and re-centrifuged three times with 5.0 ml of a 10% TCA wash solution containing 10.0 mM leucine. Following the final centrifugation, the remaining pellet was dissolved in 0.4 ml 0.5 N NaOH at $36^{0}$C. Aliquots were taken in duplicate for protein determination and radioactive counting.

SOLUBLE FRACTION

Ten percent SDS was added to the supernatant collected from the initial centrifugation process of the mitochondrial preparation to a final concentration of 0.5%. This was then left to stand at room temperature for 10 minutes. Following this, 100% (x/v) TCA was added to the solution to a final concentration of 20% and left to stand on ice for 15 minutes.

The resulting acid precipitated proteins were isolated by centrifugation at 1000 x g for 10 minutes (clinical centrifuge). The pellet obtained was washed and re-centrifuged three times with 5.0 ml of 10% TCA wash containing 10 mM leucine. Following the final centrifugation the remaining pellet was dissolved in 0.4 ml 0.5 N NaOH at $36^{0}$C. Aliquots were taken in duplicate for protein determination and radioactive counting.

F RADIONUCLIDE COUNTING PROCEDURE AND PROTEIN DETERMINATION

The tritium activity of circulating blood was assessed by counting 20ml of serum in 10.0 ml of liquid scintillation cocktail (Aquasol II) and 0.75 ml of distilled water. The results were expressed as total disintegration per minute (dpm/20 ml).

In order to determine the $^{3}$H leucine uptake into the various protein fractions of the heart, the following procedures were carried out. To a volume of 0.3 ml of dissolved proteins from the total and myofibrillar-nuclear fractions, and a volume of 0.1 ml of the mitochondrial and soluable fractions respectively, 10 ml of Aquasol II and 0.75 ml of distilled water were added.

The samples for the measurement of L-(4,5,$^{3}$H)-leucine specific activity were placed in 22.0 ml borosilicate disposable scintillation vials and counted in a Beckman LS 200 liquid scintillation counter. All determinations were

completed in duplicate. The results expressed as specific activity (S.A.) in dpm/mg of protein of each sample.

Protein content of the various heart muscle fractions were measured in duplicate utilizing a modified Biuret technique (Appendix D) with bovine serum albumin (Sigma Chemical Corp.) as a standard.

## G EXPERIMENTAL PROTOCOL AND DATA ANALYSIS

The seventeen groups of experimental animals and their treatments utilized in this study are described in Table III.

Animals designated as sedentary control (SED.C.) received normal handling but no formal exercise training. They provided data on control heart muscle protein metabolism.

Animals assigned to aerobic (AE. ACC.) and anaerobic (AN. ACC.) acclimiated groups underwent their respective performance acclimation program but were not subjected to the performance task prior to sacrifice. They provided data on the effects of the performance acclimation program on heart muscle metabolism.

Animals in the aerobic and anaerobic groups (AE. and AN. 0, 12, 24, 36, 48, 60, and 72 hours) were sacrificed at the conclusion, and at 12 hour intervals following completion of either the aerobic or anerobic performance protocol. Data from these groups were used to assess the effects of an acute bout of sprint or endurance exercise on protein metabolism in heart muscle.

Protein metabolism of heart muscle was examined by measuring the uptake of the radioactively labelled leucine by the different muscle fractions (Total Homogenete, Myofibrillar nuclear, Mitochondrial and Soluble). The data on protein labelling was first examined by Bartlett's Chi Square (Winter 1962) test for homogeneity of variance. Where no difference was found a Two-Way Analysis of Variance (ANOVA) Fixed Effect Model (D.E.R.S. ANOV 25 Program) was used. Group means were compared to give main effects for exercise treatment (Aerobic, Anaerobic) referred to as Factor A, Time (hours) (SED.C., ACC.C., and 0, 12, 24, 36, 48, 60 and 72 hours after completing the performance protocol referred to as Factor B, and interaction (AB) of the data from each fraction. Post hoc procedures involved Scheffe's multiple comparisons of main effects (1959).

Significant differences were accepted if the alpha level P is less than 0.05, where P is the probability that no difference exists between means.

To ascertain whether any differences existed between blood $^{3}$H-Leucine levels in animals of each group, a Two-Way ANOVA Fixed Effect Model was applied to serum radioactivity data (dmp/20$\mu$l. serum), an alpha level where $P \leq 0.05$ was used.

Finally the methodological reliability of the experimental technique was checked by applying a T-test (Ferguson, 1966) to the duplicate results obtained for protein concentration and radioactivity of the same fraction of muscle taken from five randomly selected animals. An alpha level where $P \leq 0.05$ was used.

# III RESULTS

The descriptive and statistical analysis of the results are presented under the following headings: I) Tritiated Leucine Incorporation Into Protein, II) Methodological Reliability and III) Statistical Significance.

An abridgement of the experimental results is presented in graph form. The pertinent data for all experimental animals is presented in Tables XI and XII, Appendix C.

## I. TRITIATED LEUCINE INCORPORATION INTO PROTEIN.

In figures 1-10 the acute effects of two forms of exercise on protein synthesis within the various heart muscle fractions are plotted against time.

Figures 1-4 demonstrate the amount of tritiated leucine incorporated into the whole muscle homogenate, myofibrillar-nuclear, mitochondrial and soluble fractions plotted against the time elapsed after completion of the anaerobic exercise for the sedentary, control and acclimated animals. The tritiated leucine is expressed as relative specific activity (S.A.) is dpm/mg of protein.

The effects of an acute bout of aerobic exercise on the aforementioned are shown in figures 5-9.

The differential effects of exercise on the separate heart fractions following either an anaerobic or aerobic exercise stimuli are demonstrated in figures 9 and 10.

Figures 11-13 allow the comparison of effects of the areobic and anaerobic exercise stress on the level of leucine incorporation within the same fraction.

A. Effects of Exercise Acclimation

Comparison of the sedentary control (sc) and the acclimated control (ac), reveals the effects of the acclimation program on leucine incorporation. This comparison in figures 2, 3 and 4 demonstrate the effects of the anaerobic acclimation program on the myofibrillar-nuclear, mitochondrial and soluble fractions. The level of leucine incorporation was lowered in all fractions as a result of the anaerobic acclimation program. The effects of the aerobic acclimation program on leucine incorporation into the three heart fractions are found in figures 6, 7 and 8. The aerobic program appears to produce the same effect as the anaerobic program, that is, leucine incorporation was reduced in all fractions in the acclimated animals as compared to the sedentary control levels. The differences between the acclimation programs and the magnitude of reduction in leucine uptake in the three heart fractions, are found in figures 11-13. Figures 11 and 13 demonstrate that the aerobic acclimation program results in a greater reduction in leucine uptake in the myofibrillar-nuclear and soluble fractions. Figure 12 demonstrates that there was no difference in the level of reduction in leucine uptake in the mitochondrial fraction as a result of the different acclimation programs.

B. Immediate Effects of Exercise

The immediate effects of either an anaerobic or aerobic exercise stimuli on the level of leucine incorporation are found in comparing the acclimated control (ac) and the 0

hour values. This comparison in figures 2, 3 and 4 demonstrates the immediate effects of anaerobic exercise on protein uptake in the myfibrillar-nuclear, mitochondrial and soluble fractions of the heart. The response in the mitochondrial and soluble fractions was a slight decrease in protein labelling. The initial response was not consistent in all fractions as evidenced by the slight increase in protein labelling in the myofibrillar-nuclear fraction. In figures 6, 7 and 8 the immediate effects of aerobic exercise on leucine incorporation are demonstrated. Aerobic exercise results in an increased leucine incorporation in all three heart fractions. Figures 11-13 demonstrate differences between the anaerobic and aerobic exercise stimuli and their immediate effect on protein uptake patterns in the three heart fractions. The variable response patterns between fractions produced by anaerobic exercise contrasts the consistency in the response patterns produced by aerobic exercise.

C. Post Exercise Effects

The post exercise response patterns for leucine incorporation are demonstrated by the 12-72 hour values.

i Myofibrillar-nuclear Fraction

The time course following anaerobic exercise for the myofibrillar-nuclear fraction is found in figure 2. The group average or mean data indicates that following the immediate increase in leucine incorporation in this fraction, the activity steadily decreases to 36 hours post exercise.

Examination of the individual results at the 12 hour interval, reveals that the leucine uptake in two of the three animals was higher than the mean 0 hour level. The mean decrease in leucine incorporation demonstrated at the 12 hour interval was likely produced by an atypical response in one animal. It is probable then, that the leucine uptake is either further elevated or plateaus 12 hours post exericse, rather than the decline in activity observed when the results from all three animals are considered. The protein labelling reached its lowest level 36 hours post exercise. At that time there was a sharp increase in protein labelling peaking at 48 hours. The activity then returned to below baseline levels at 60 hours. The mean data indicates that there was a slight increase in leucine incorporation 72 hours post exercise. This response was similar to what occurred at the 12 hour interval. That is, the mean data is biased by an atypical response in one animal. The leucine incorporation was lower than the mean 60 hours level in two of the three animals. Thus, it is likely that there was a further decrease in activity 72 hours post exercise.

The time course following aerobic exercise for the myofibrillar-nuclear fraction is found in figure 6. The intergroup variability in the leucine uptake observed in the anaerobic animals was also seen in the aerobic animals. At the time intervals 12, 36 and 60 hours post exercise, the mean data plot is being influenced by aberrant responses in one of the three animals. The mean data response pattern demonstrates

a slight increase in activity 12 hours post exercise. This was followed by a steady decline in activity to 36 hours. From that point leucine uptake increased to 48 hours and then decreased steadily to 72 hours post exercise. The curve produced when the aberrant responses are taken into consideration is substantially different. Rather than the increase in protein uptake at 12 hours there is a plateau slightly decreasing to 24 hours post exercise. At this time, in sharp contrast to the mean data, the activity increases peaking at 36 hours. From this point the activity falls to 48 hours. At the 60 hour mark the protein labelling plateaus or slightly increases and then decreased to 72 hours post exercise.

Figure 11 allows the time course patterns between the anaerobic and aerobic groups to be compared. The levels of $^{3}H$-leucine incorporation into the myofibrillar-nuclear fraction following acute exercise, suggest that anaerobic and aerobic exercise differentially affect protein incorporation. It appears that the post exercise response was of greater magnitude following anaerobic exercise. Also, the response occurs at a later time following anaerobic exercise (48 hours) as compared to aerobic exercise (36 hours).

ii Mitochondrial Fraction

The time course pattern for leucine incorporation following anaerobic exercise in the mitochondrial fraction is found in figure 3. The mean data demonstrates a decrease in the leucine uptake at the 12 hour interval. This was followed by a slight increase in the activity 24 hours post exercise.

This increase is produced by an atypical response in a single animal. In all likelihood, the protein labelling steadily drops reaching its lowest level 36 hours post exercise, thereafter activity sharply increased peaking at 48 hours. From this point protein labelling rapidly falls to the 60 hour mark following which there was an increase 72 hours post exercise.

The effects of an aerobic exercise stimuli on the time course response in the mitochondrial fraction is found in figure 7. The activity steadily decreased to 24 hour post exercise. At this time there is an increase in protein labelling which was followed by a slight decrease at the 48 hour mark. Considering the fact that in two of the three animals protein labelling is increasing at this time, it is reasonable to expect the activity was either plateaued or further increased 48 hours post exercise. From this point the activity fell to the 60 hour mark following which there was an increase 72 hours post exercise.

Figure 12 allows the time course patterns between the anaerobic and aerobic groups to be compared. The comparison in this fraction (mitochondrial) is similar to that found in the myofibrillar-nuclear fraction. The increase in leucine uptake was more pronounced and delayed following exposure to the anaerobic exercise as compared to aerobic exercise.

iii Soluble Fraction

The time course pattern for leucine incorporation following anaerobic exercise into the soluble fraction is found in figure 4. The increase in protein labelling at 12

hours was followed by a steady decrease in activity reaching its lowest level 36 hours post exercise. The activity then sharply increased reaching its highest level at 48 hours. From this point leucine uptake dropped continually to the 72 hour mark.

The effects of an aerobic exercise stimuli on the time course response in the soluble fraction is found in figure 8. The activity steadily dropped to 24 hours post exercise. At this time there was a sharp increase in protein labelling reaching its highest peak at 36 hours. From this point the activity decreased to the 48 hour mark. There was a slight increase in activity 60 hours post exercise which was followed by a drop in protein labelling at the 72 hour mark.

Figure 13 allows the time course patterns between the anaerobic and aerobic groups to be compared. The difference between exercise effects in the soluble fractions was similar to that demonstrated in the previous two fractions. That is, the response was more prominent and postponed in the anaerobic animals.

iv Differences Between Fractions

Figure 9 allows for comparison in the protein uptake patterns to be made between the myofibrillar-nuclear, mitochondrial and soluble fractions of the anaerobic animals. The responses are quite consistent between the different fractions. That is, the time intervals where the lowest (36 hours) and highest (48 hours) levels of activity were attained are the same for all fractions measured. Differences in the

response patterns occurred at 12 and 72 hours post exercise. The activity 12 hours post exercise was increasing in the myofibrillar-nuclear and soluble fractions. This contrasts the decrease in protein labelling found in the mitochondrial fraction at this time. Protein labelling 72 hours post exercise was decreasing in the myofibrillar-nuclear and soluble fractions and was increasing in the mitochondrial fraction.

The differences in the patterns of protein uptake for the heart fractions following aerobic exercise are found in figure 10. The response patterns in the aerobic animals do not demonstrate the same degree of harmony that was evident in the anaerobic animals. The highest level of activity occurred immediately following exercise in the mitochondrial fraction. While peak levels of protein uptake did not occur until 36 hours post exercise in the myofibrillar-nuclear and soluable fractions. The lowest levels of activity for the myofibrillar-nuclear, mitochondrial and soluble fractions occurred 72, 60 and 24 hours post exercise. Differences between the response patterns were evidenced at the 48 and 60 hour intervals. The activity 48 hours post exercise decreased in the myofibrillar-nuclear and soluble fractions, and increased in the mitochondrial fraction. At 60 hours post exercise protein labelling decreased in the myofibrillar-nuclear and mitochondrial fractions. The soluble fraction demonstrated an increase in activity at this time.

II. METHODOLIGICAL RELIABILITY

The reliability coefficients for the duplicate measures of protein concentration and radionuclide counting on the different

heart fractions are presented in Table IV. The correlations expressed in this table indicate a high degree of consistency in the results produced by the experimental techniques utilized for protein and radioactivity determinations.

## III. STATISTICAL SIGNIFICANCE

Homogeneity of variance in the data is critical for meaningful application of the Analysis of Variance test. Results from the Chi Square test for Homogeneity of Variance applied to the $^{3}$H-leucine incorporation results, revealed that this data does not comply with homogeneity of variance restrictions. Because of this heterogeneity the Analysis of Variance tests was judged not suitable and hence will not be presented. For discussion purposes the trends evident in the graphs (Figures 1-13) will be utilized.

Analysis of Variance in blood serum radioactivity data (Table V) show that no difference existed among blood $^{3}$H-leucine pools in all animals of the different experimental groups.

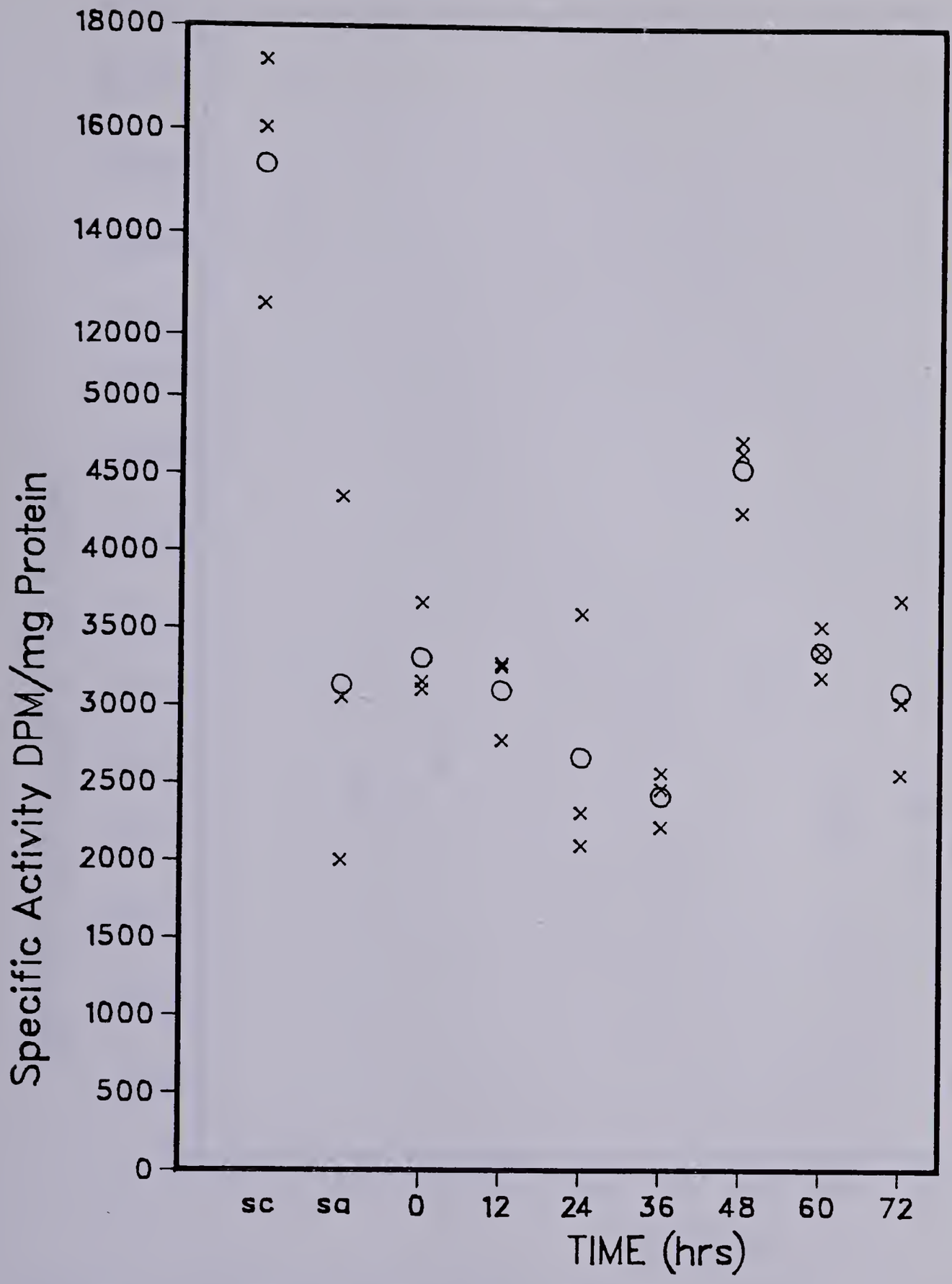


Figure 1. Incorporation of the radionuclide from L-(4,5 $^{3}$H)-leucine into protein in the total homogenate fraction of the heart muscle from animals of the anaerobic group (SC-sedentary control; AC-acclimated control). The graph is composed of the group mean ○ and individual animal data ×.

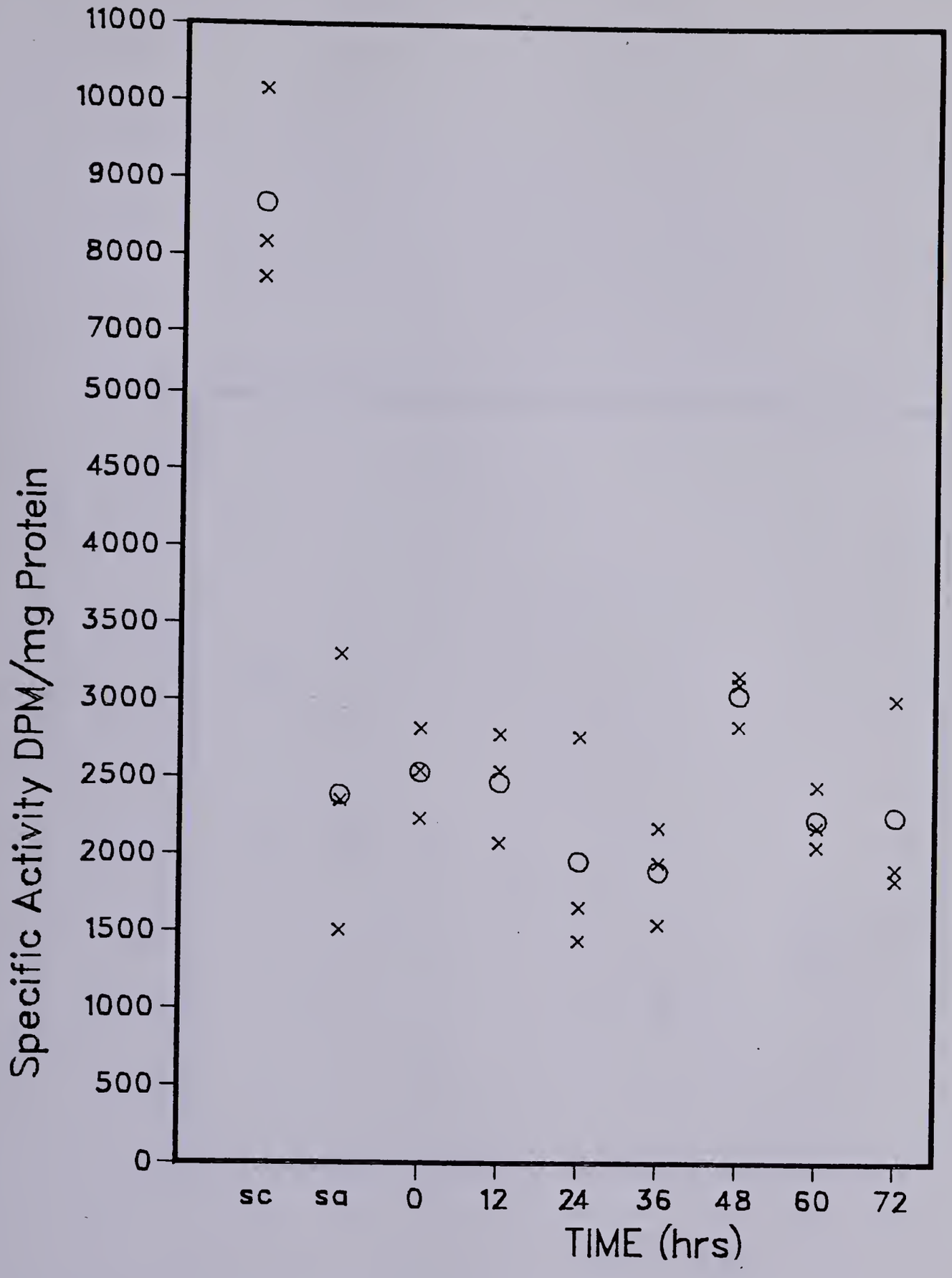


Figure 2. Incorporation of the radionuclide from L-(4,5 $^{3}$H)-leucine into protein in the myofibrillar-nuclear fraction of the heart muscle from animals of the anaerobic group (SC-sedentary control; AC-acclimated control). The graph is composed of the group mean ○ and individual animal data ×.

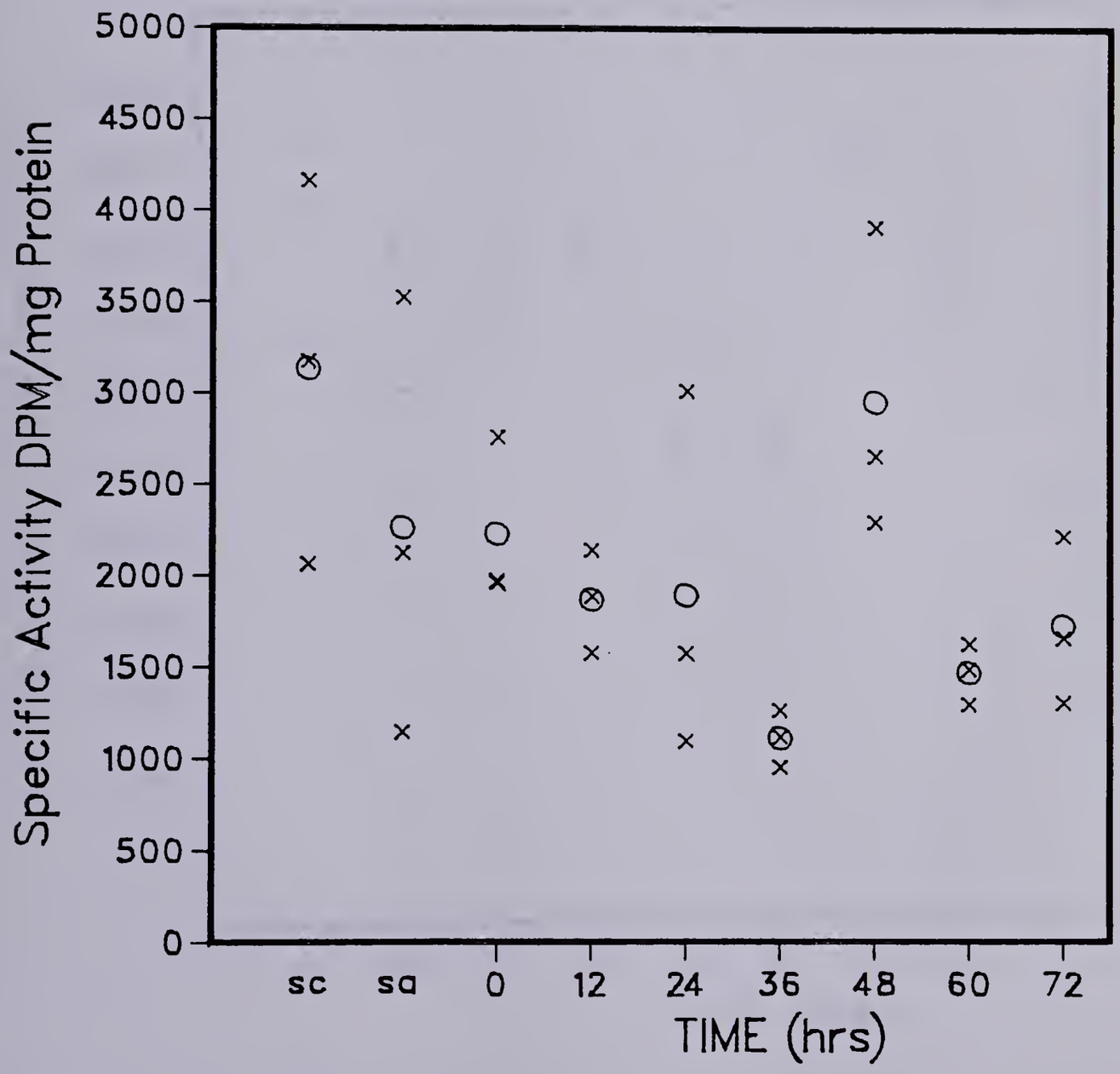


Figure 3. Incorporation of the radionuclide from L-(4,5 $^{3}$H)-leucine into protein in the mitochondrial fraction of the heart muscle from animals of the anaerobic group (SC-sedentary control; AC-acclimated control). The graph is composed of the group mean ⊗ and individual animal data ×.

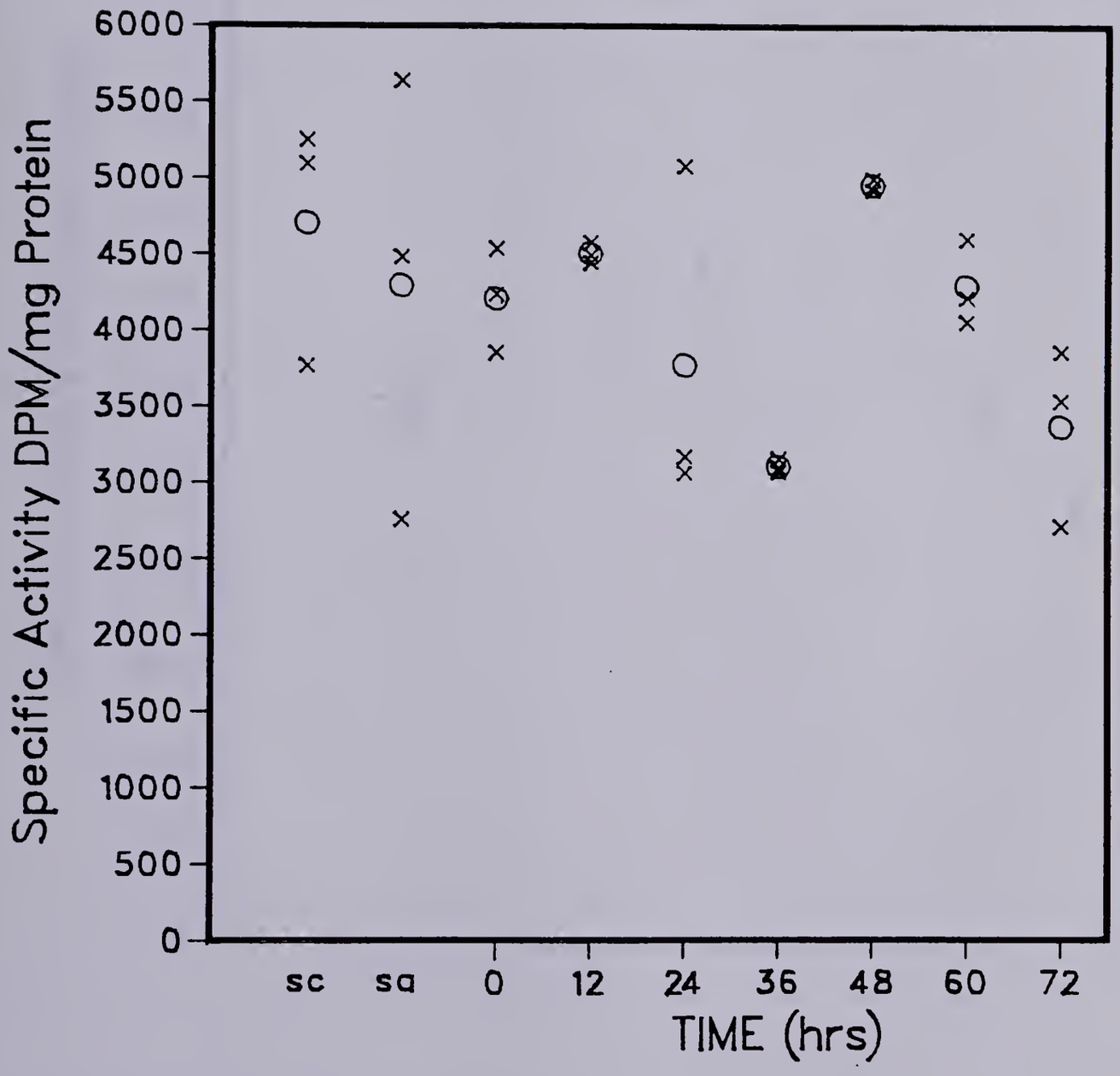


Figure 4. Incorporation of the radionuclide from L-(4,5 $^{3}$H)-leucine into protein in the soluble fraction of the heart muscle from animals of the anaerobic group (SC-sedentary control; AC-acclimated control). The group is composed of the mean O and individual animal data ×.

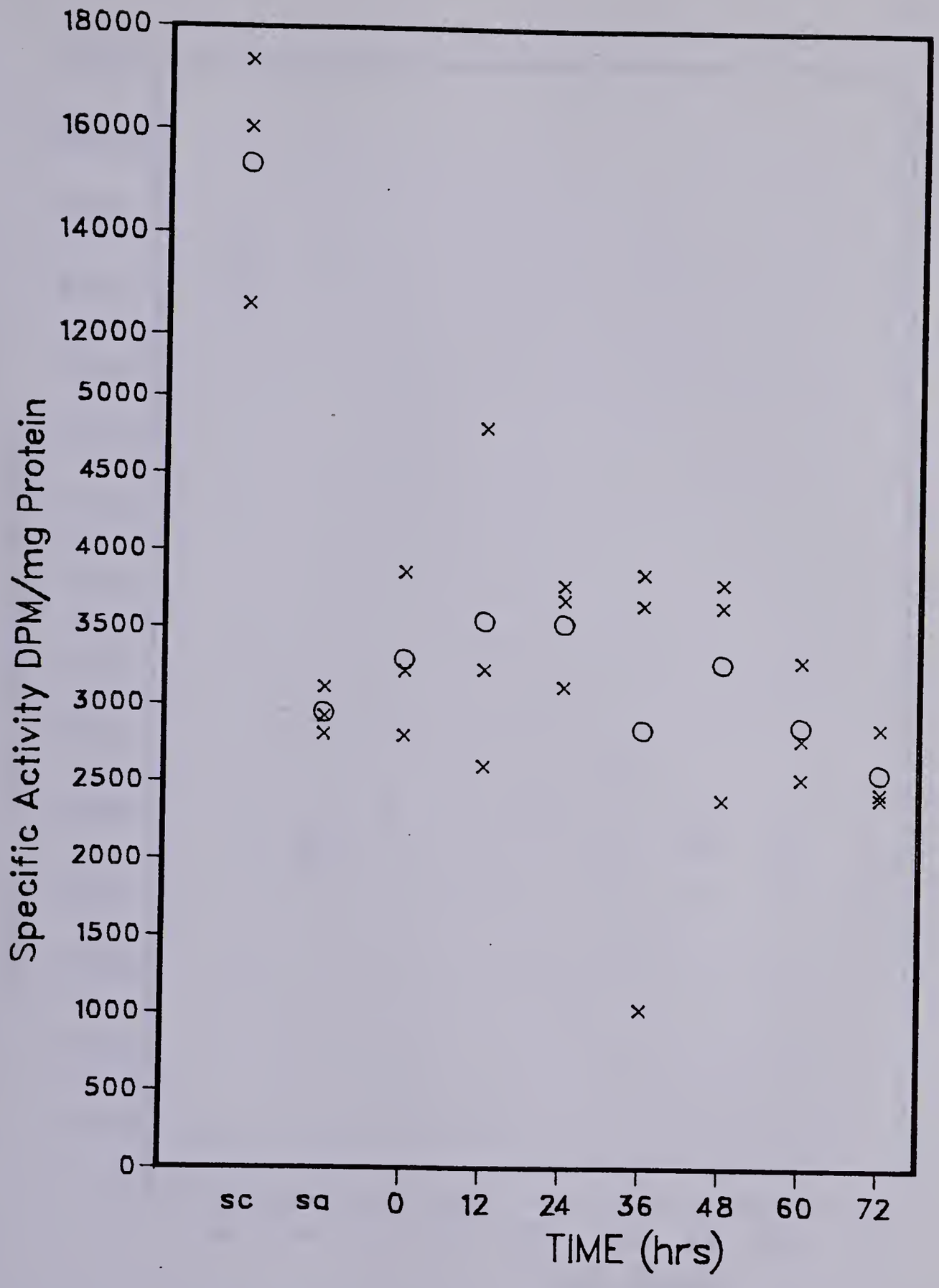


Figure 5. Incorporation of the radionuclide from L-(4,5 $^{3}$H)-leucine into protein in the total homogenate fraction of the heart muscle from animals of the aerobic group (SC-sedentary control; AC-acclimated control). The group is composed of the group mean ○ and individual animal data ×.

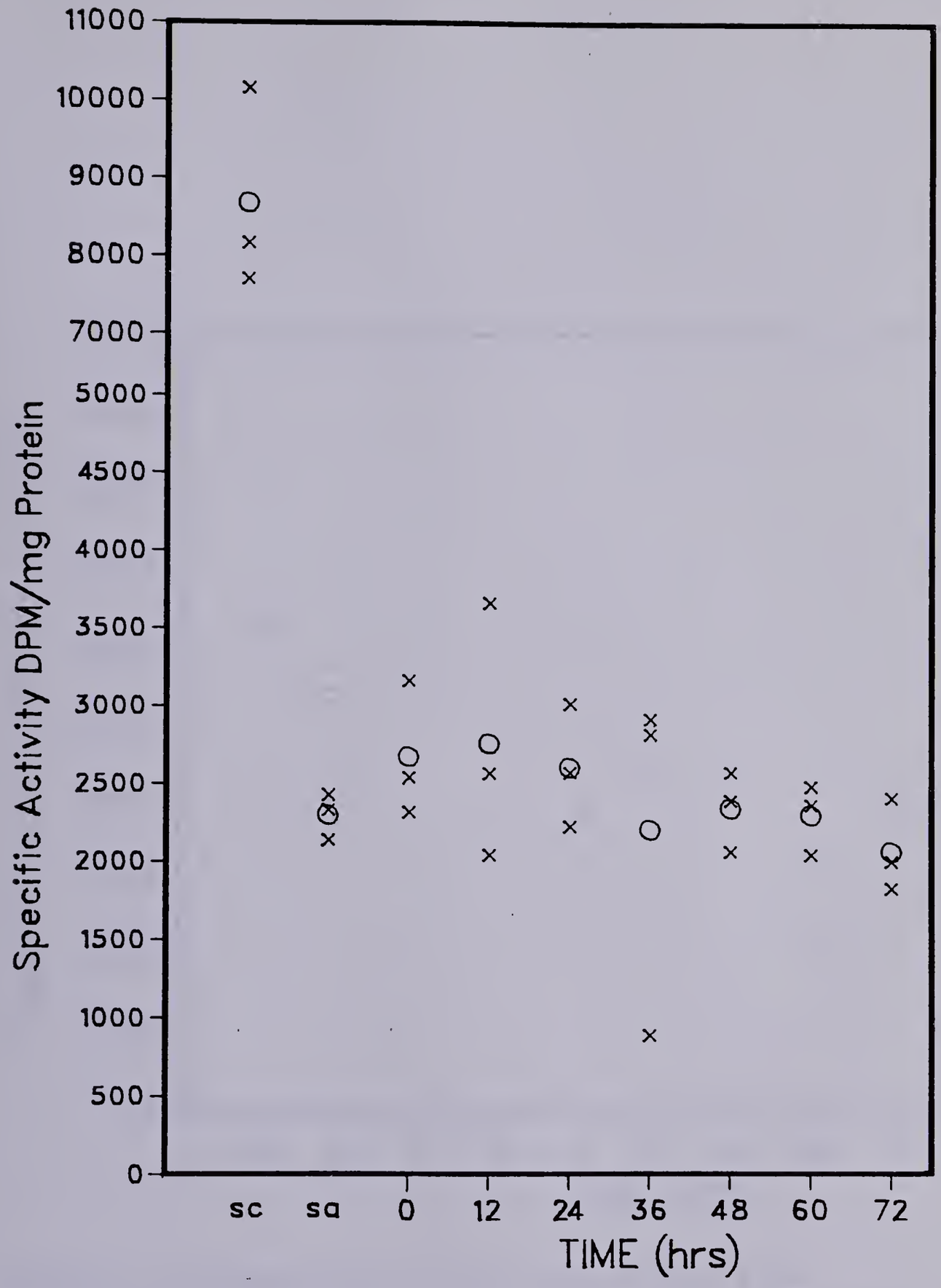


Figure 6. Incorporation of the radionuclide from L-(4,5 $^{3}$H)-leucine into protein in the myofibrillar-nuclear fraction of the heart muscle from animals of the aerobic group (SC-sedentary control; AC-acclimated control). The graph is composed of the group mean ○ individual animal data ×.

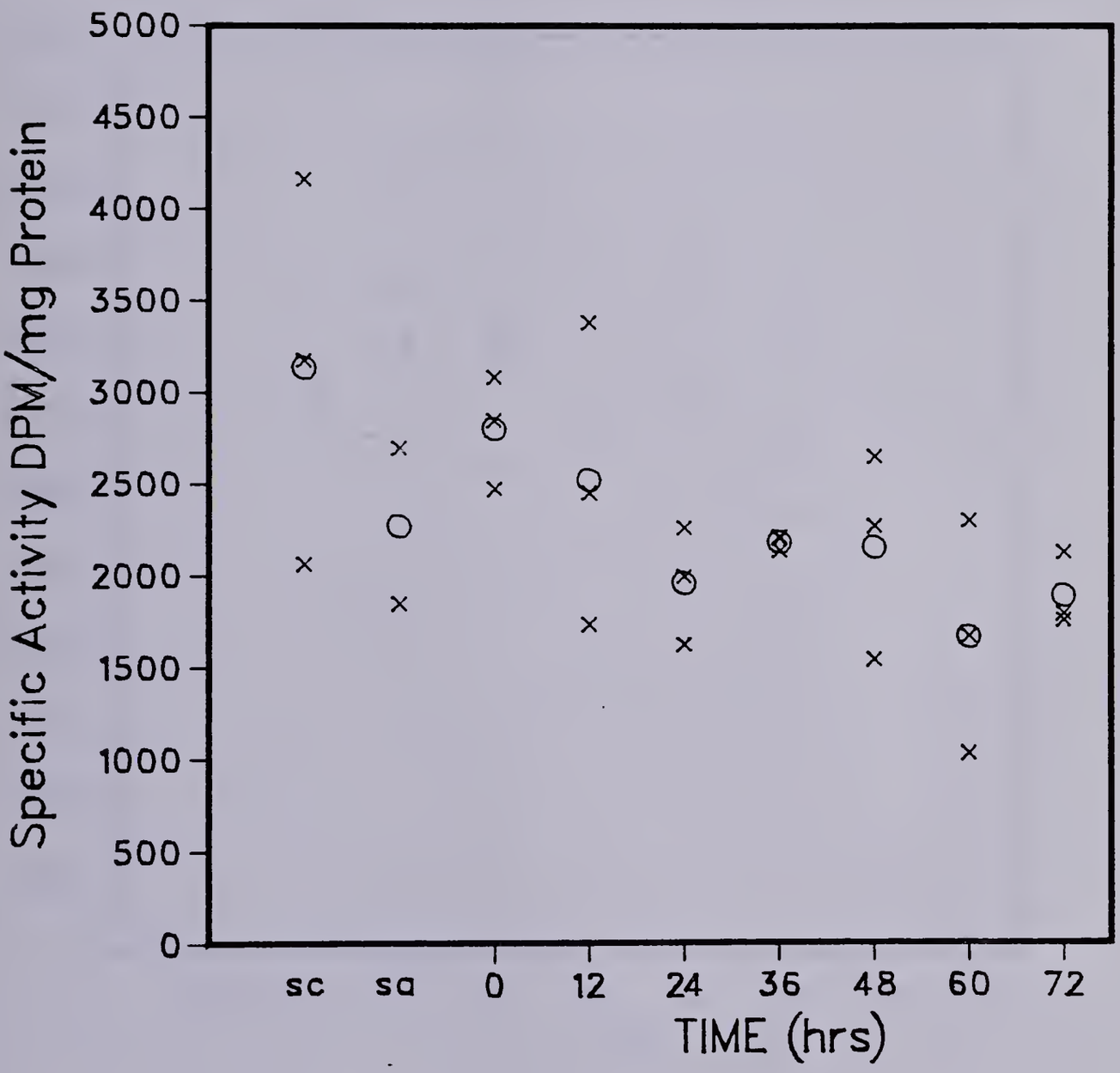


Figure 7. Incorporation of the radionuclide from L-(4,5 $^{3}$H)-leucine into protein in the mitochondrial fraction of the heart muscle from animals of the aerobic group (SC-sedentary control; AC-acclimated control). The graph is composed of the group mean ⊙ and individual animal data ×.

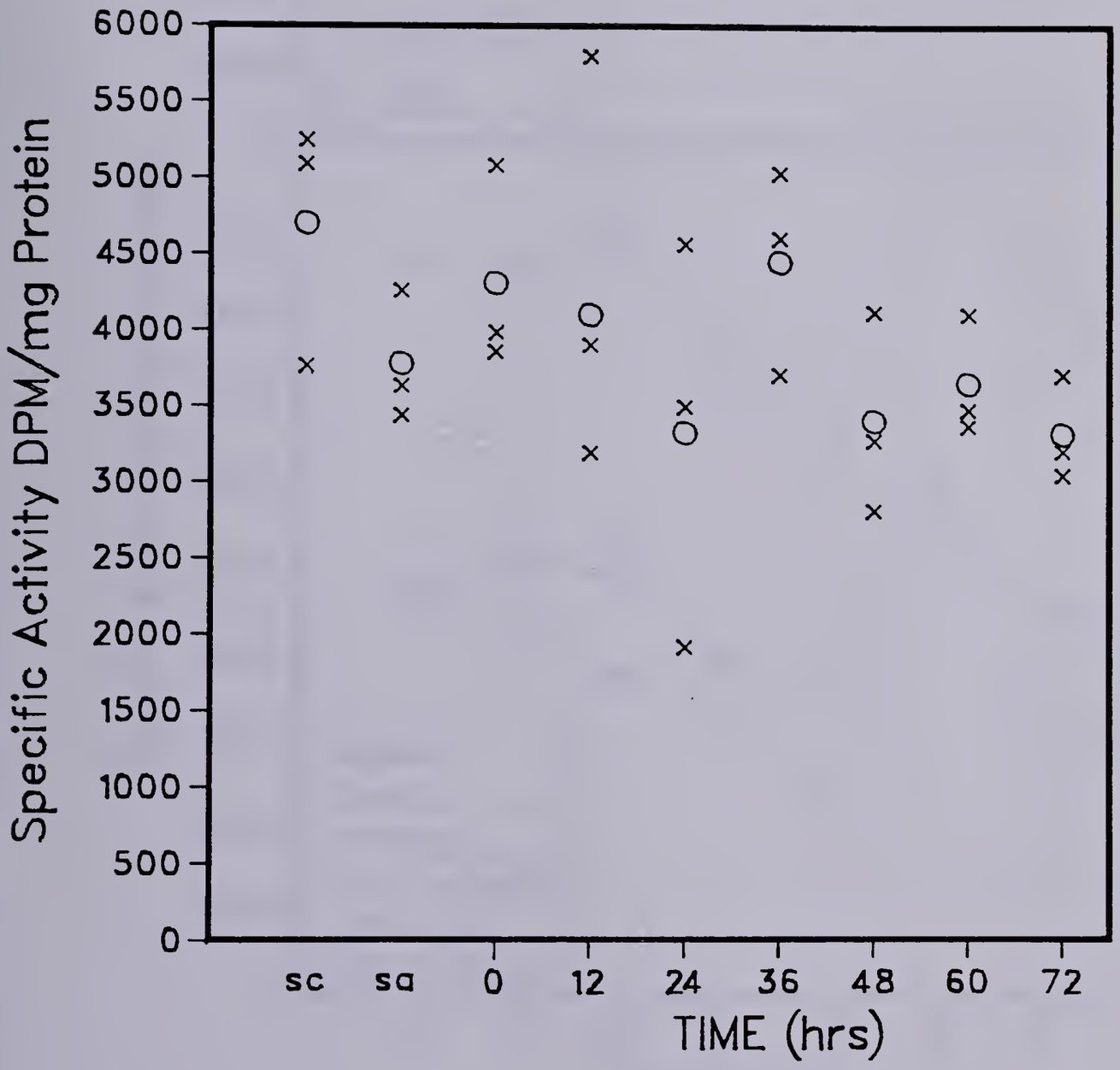


Figure 8. Incorporation of the radionuclide from L-(4,5 $^{3}$H)-leucine into protein in the soluble fraction of the heart muscle from animals of the aerobic group (SC-sedentary control; AC-acclimated control). The graph is composed of the group mean O and individual animal data ×.

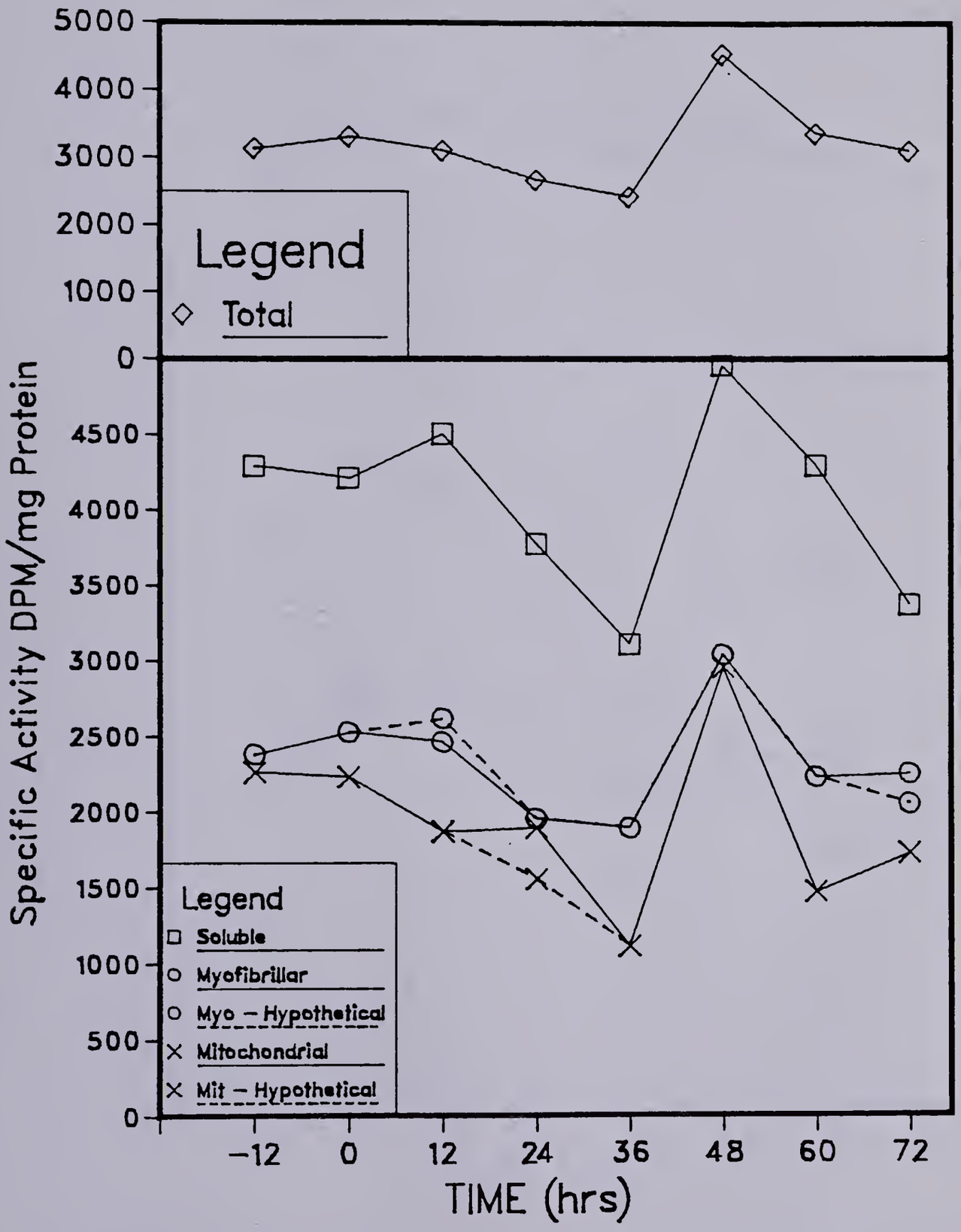


Figure 9. Incorporation of the radionuclide from L-(4,5 $^{3}$H)-leucine into protein in all fractions of the heart muscle from animals of the anaerobic groups (AC-acclimated control). The graph is composed of the group mean data.

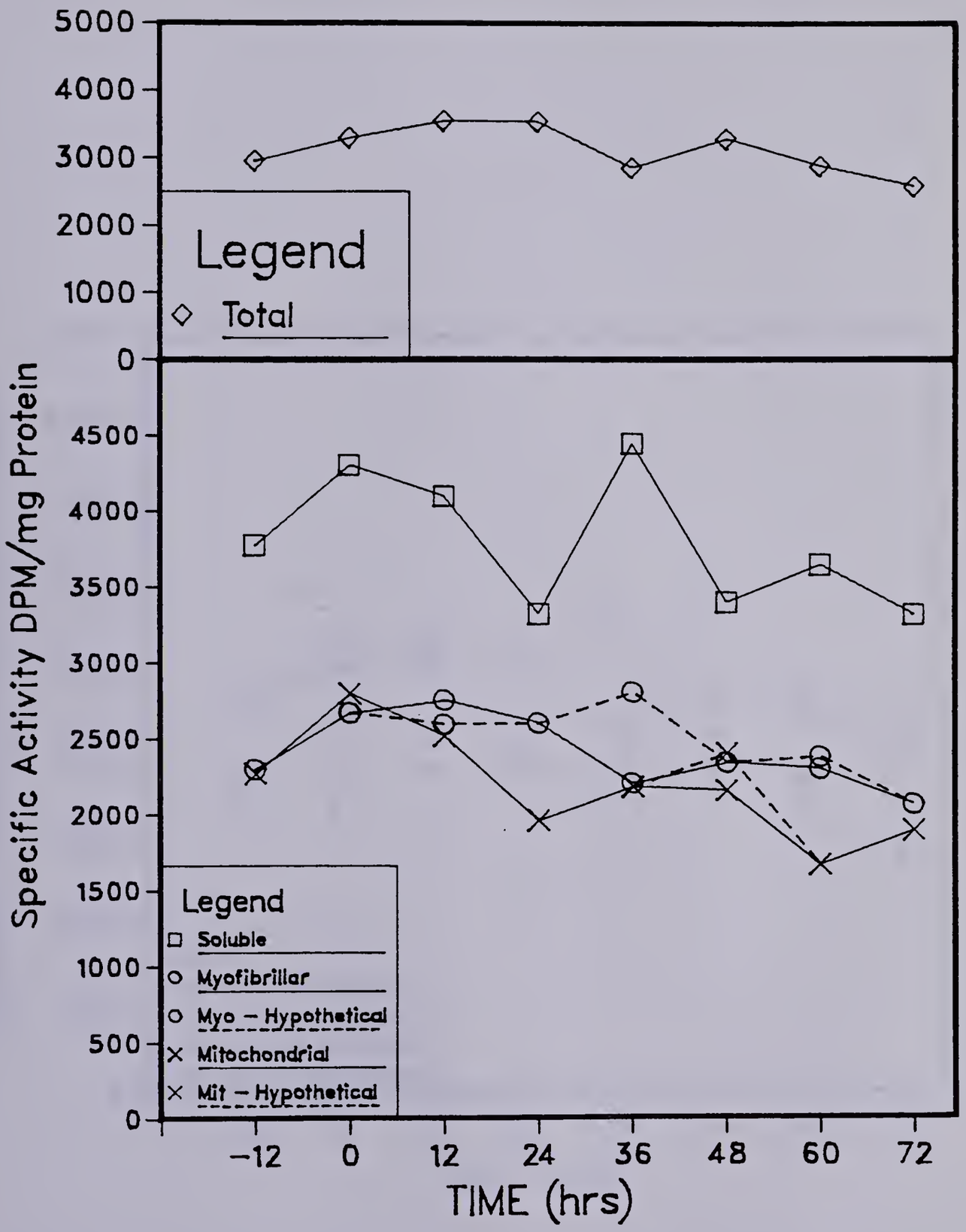


Figure 10. Incorporation of the radionuclide from L-(4,5 $^{3}$H)-leucine into protein in all fractions of heart muscle from animals of the aerobic groups (AC-acclimated control). The graph is composed of group mean data.

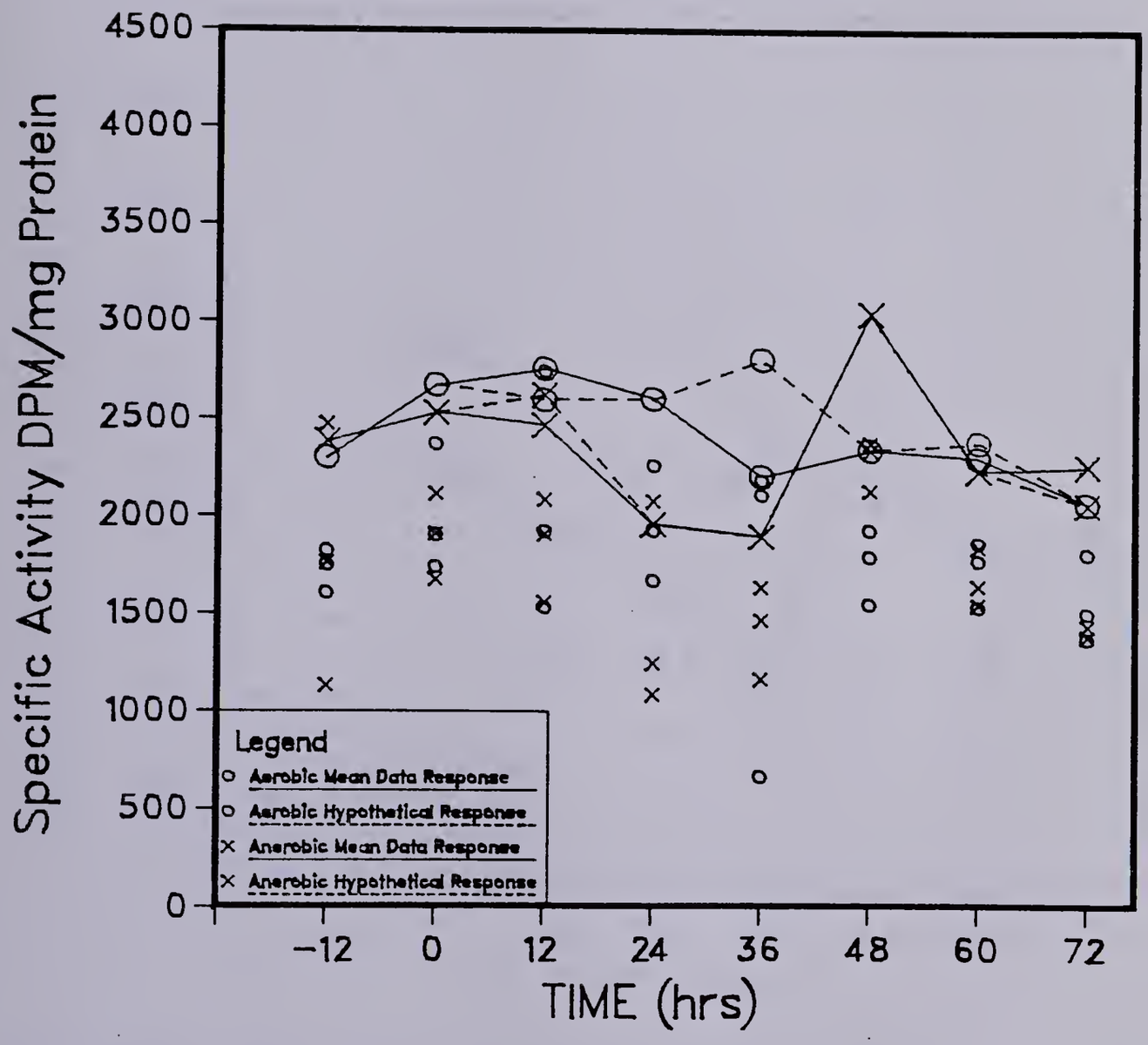


Figure 11. $^{3}$Incorporation of the radionuclide from L-(4,5 $^{3}$H)-leucine into protein in the myofibrillar fraction of heart muscle from animals of the aerobic and anerobic groups (AC-acclimated control). The graph is composed of group mean O and individual animal data ×.

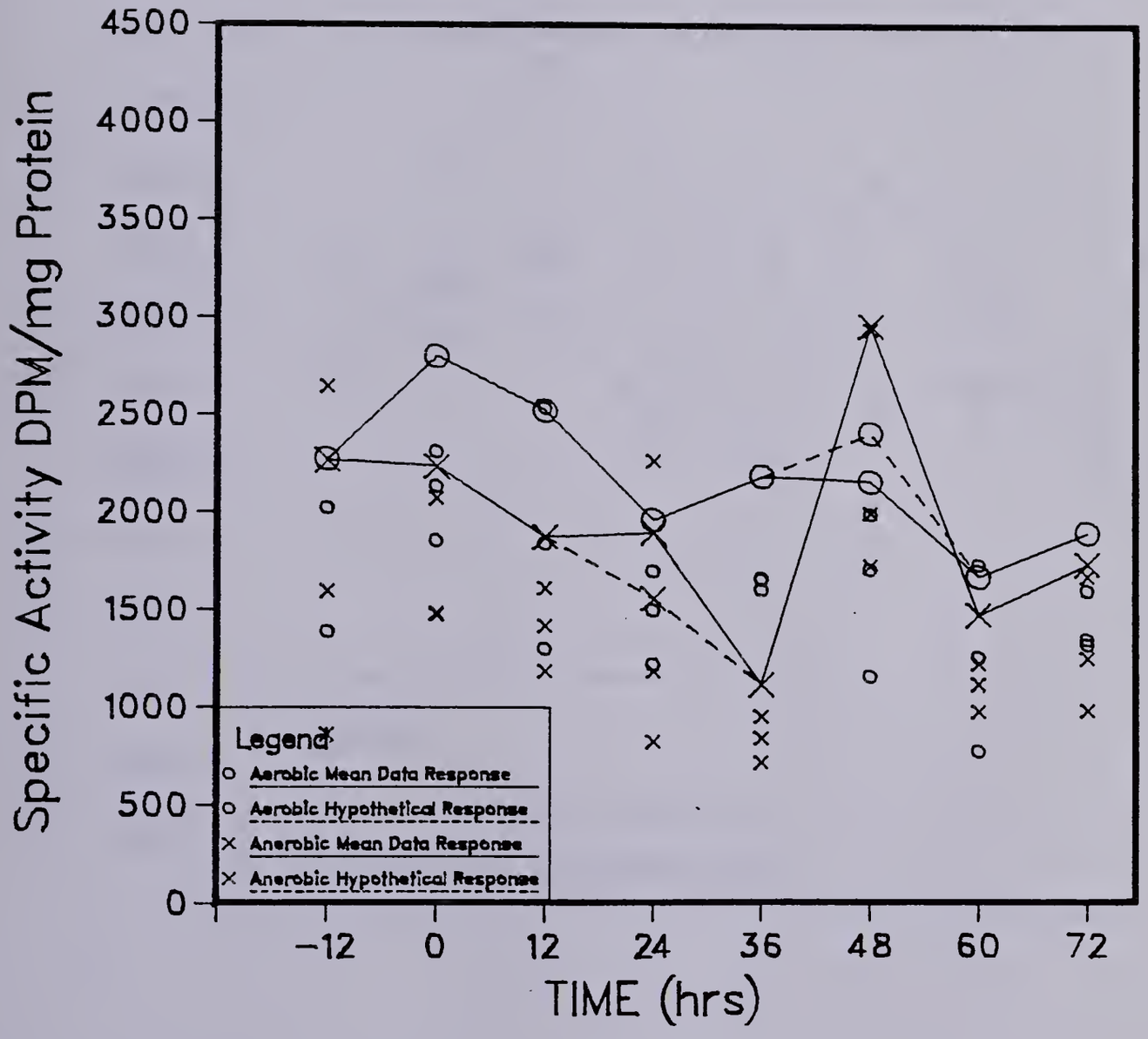


Figure 12. Incorporation of the radionuclide from L-(4,5 $^{3}$H)-leucine into protein in the mitochondrial fraction of the heart muscle from animals of the aerobic and anaerobic groups (SC-sedentary control; AC-acclimated control). The graph is composed of group mean O and individual animal data ×.

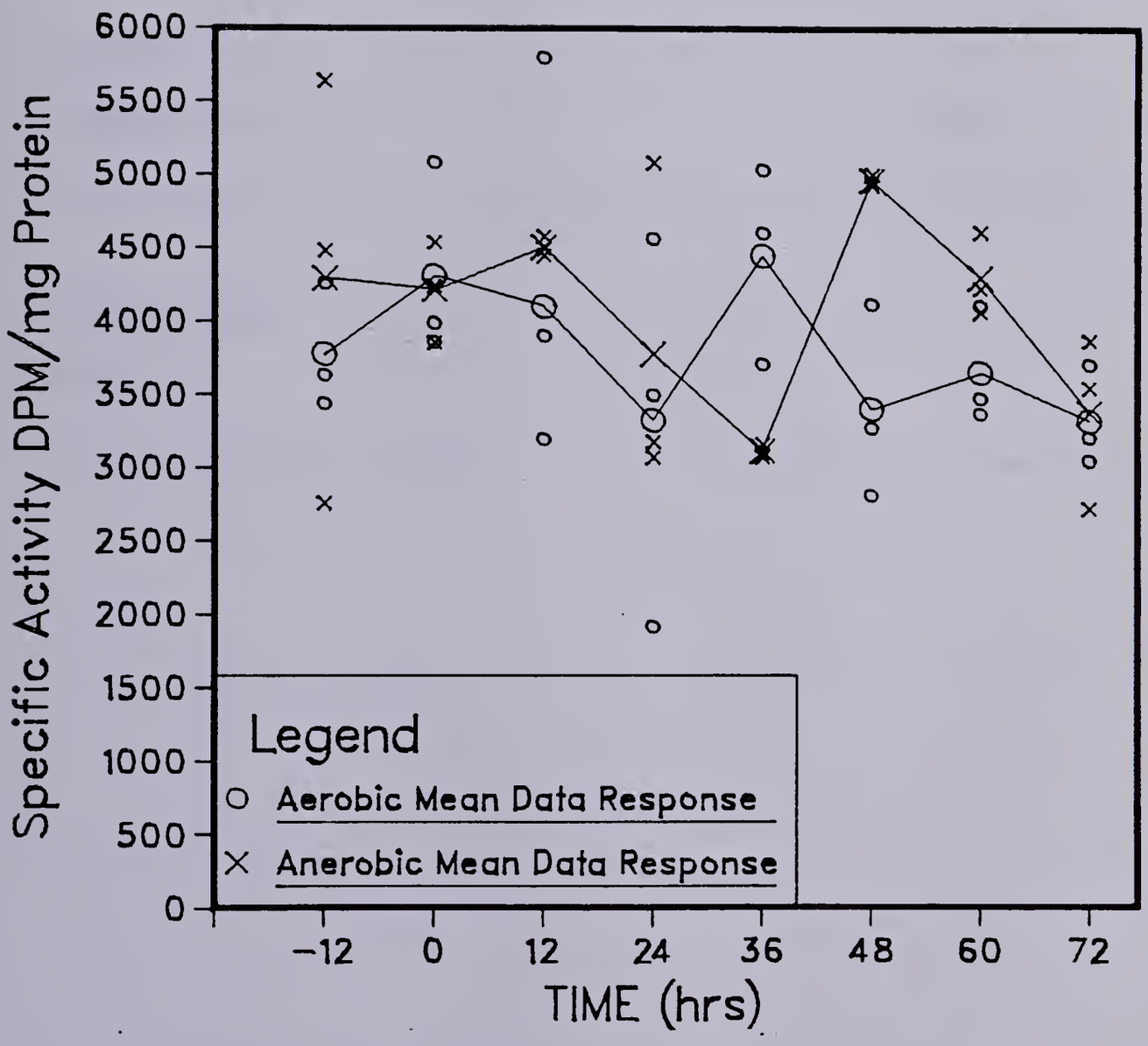


Figure 13. $_{3}$Incorporation of the radionuclide from L-(4,5 $^{3}$H)-leucine into protein in the soluble fraction of the heart muscle from animals in the aerobic and anerobic groups (SC-sedentary control; AC-acclimated control). The graph is composed of group mean O and individual animal data ×.

Table IV Reliability coefficients between duplicate aliquots for, protein determination and radionuclide counting, in the myofibrillar-nuclear, mitochondrial and soluble fractions from all animals.

| Experimental Procedure | Myofibrillar-nuclear Fraction | Mitochondrial Fraction | Soluble Fraction |
|---|---|---|---|
| Protein Concentration | .95 | .98 | .99 |
| Radionuclide Counting | .99 | .98 | .99 |

Table V Analysis of Variance table for blood serum $^{3}H$-leucine pools in all animals.

ANALYSIS OF VARIANCE TABLE

| SOURCE | SUM OF SQUARES | D.F. | MEAN SQUARES | F RATIO | PROBABILITY |
|---|---|---|---|---|---|
| A* | 0.852E+07 | 1 | 0.852E+07 | 3.286 | 0.076247 |
| B | 0.357E+08 | 8 | 0.446E+07 | 1.721 | 0.118332 |
| AB | 0.173E+08 | 8 | 0.217E+07 | 0.809 | 0.599242 |
| E | 0.105E+09 | 39 | 0.268E+07 | | |
| TOTAL | | 56 | | | |

*A - Treatment Effect

B - Time Effect

AB - Interaction

HOMOGENEITY OF VARIANCE TEST CHISQ=0.154E+02

PROBABILITY=0.57045

# IV DISCUSSION

The following discussion is an attempt to explain and integrate the changes observed in leucine incorporation in response to anaerobic and aerobic exercise. The discussion is presented under the major headings: 1) Methodological Reliability, 2) Tritiated Leucine Incorporation into Protein, 3) Practical Implications and 4) Recommendations for Future Study.

## I. METHODOLOGICAL REALIABILITY

An examination of the leucine incorporation data illustrated in figures 1-13 reveals that, 1) an exercise acclimation program alters the rate of leucine incorporation, 2) there is a distinct difference in the response patterns following an anaerobic as compared to an aerobic exercise session, 3) there is a reasonably large amount of inter group variability in leucine incorporation. Prior to the discussion it is important to describe the experimental procedures utilized to ensure that the observed differences in the data were in fact due to variable biological responses, and not due to error in methodological techniques.

Realiability coefficients between the independently processed duplicate samples for protein determination and radio nuclide activity were calculated for the separate heart fractions (Table IV). The valves indicate that these analytical techniques were reliable and were not the cause for the differences in the data.

The Analysis of Variance carried out on the serum radioactivity data (Table V) showed that there was no

significant difference in the serum $^{3}$H-leucine levels between the different groups. This would suggest that variability in the circulating $^{3}$H-leucine precursor pool was not responsible for the differences seen in the protein labelling data.

The sacrifice schedule used (Table XIII) was designed to ensure that the time of sacrifice for each animal within an experimental group occurred at different times of the day, i.e. rats were not sacrificed at any particular time of their diurnal cycle. There was no apparent relationship between the animals time of sacrifice and the $^{3}$H-leucine incorporation into the different heart fractions. Thus it is unlikely that any diurnal variation in protein metabolism was responsible for the differences in the protein labelling data.

Finally, since the trends evident in leucine incorporation were obtained utilizing a random sacrifice schedule, it is unlikely that the order of sacrifice was the cause of the differences in protein labelling.

Considering the procedures taken to guard against methodological error, it becomes apparent that the trends evident in the protein labelling data were not caused by methodological error. It is therefore reasonable to conclude that the differences in leucine incorporation demonstrated in the heart were the result of a differential responses in protein metabolism. And that the differential responses are being produced by the exposure to either an acute anaerobic or aerobic exercise session.

The following discussion is based upon the trends evident in the leucine incorporation data. Clearly the interpetation of these trends is limited by the experimental group size, and the degree of intergroup variability in the measured response of the dependent variables. Included in the discussion are possible explanations for this intergroup variability and suggestions for methodology to reduce it.

## II. TRITIATED LEUCINE INCORPORATION INTO PROTEIN

### A. Effects of Exercise Acclimation

The most common methods used for endurance training animals are via either a swimming or treadmill running program. Both of these modes of exercise have been shown to increase cardiac size relative to body weight (Oscai et al., 1971; Dowell et al., 1976). This hypertrophy is not associated with increased protein synthetic activity. Rather, it is due to a maintenance of heart size in spite of an exercise induced decrease in body weight (Dowell et al., 1976). It has been reported that protein synthesis activity in cardiac tissue is suppressed by both a chronic endurance exercise program (Dohm et al., 1977), and an acute endurance exercise bout (Zimmer et al., 1971). This suppressed rate of protein synthesis in response to a chronic exercise stress is in agreement with the present study. Although the aerobic animals were not formally trained and only subjected to an acclimation program, it did result in a suppression of protein synthesis activity in all fractions. This decrease in protein synthesis in the different heart fractions was also demonstrated in the animals subjected to

an anaerobic acclimation program. The difference in the intensity of the acclimation programs does not appear to have a differential effect on protein synthesis. This finding is in opposition to the original experimental hypothesis that different exercise intensities would have a differential effect on leucine incorporation. The previously mentioned exercise induced cardiac hypertrophy appears to occur in spite of reduced protein synthesis activity in the organ. This indicates that chronic exercise has a greater effect on protein degradation, (i.e., a reduced degradation) rather than on protein synthesis (Dohm et al., 1977). The resultant greater ratio of heart to body weight in exercised animals is associated with an increased capacity to deliver blood to the working muscles (Oscai et al., 1971).

B. Immediate Effects of Exercise

It has been demonstrated that an acute exposure to an exhaustive bout of swimming suppressed protein synthesis activity in the total heart homogenate (Zimmer et al.,1971) and in the intra and extra mitochondrial fractions (Cook et al., 1981). Results from the present study do not support this finding. The activity in all three heart fractions measured increased immediately following the aerobic exercise bout. Probable explanations for this difference are; the inherent differences between swimming and treadmill running, and the lack of either an acclimation or training program in the studies by Zimmer and Cook. Another possible

explanation for the dissimilarity in the initial response between swimming and treadmill running may be related to the difference in intensity between the two exercise stimuli. The results from this study give support to this hypothesis. That is, the two intensities of exercise examined produced different effects on protein uptake. The activity in the mitochondrial and soluble fractions decreased following exposure to the higher intensity anaerobic exercise bout. This is the opposite effect to that produced by aerobic exercise.

C. Post Exercise Effects

The previously mentioned cardiac hypertrophy resulting from endurance training has been attributed to a reduction in protein degradation (Dohm et al., 1977). The results from the acclimated animals, (lower levels of leucine incorporation as compared to sedentary control animals), in the present study support this hypothesis. However, an examination of the response in protein metabolism over an extended time period following acute exercise yields some additional information. The results from Bostron et al., (1974), infer that protein synthesis increases in the total heart fraction 24 hours following an exhaustive swim. The present results demonstrate that the amino acid uptake increases above control levels in the different heart fractions at various times following exercise. The results from both Bostron's and the current study suggest that an increase in protein synthesis during the recovery period, may also be a factor in the production of cardiac hypertrophy.

The differential effects due to the intensity of exercise on protein synthesis found immediately following exercise, are also evident in the post exercise time course results. As evidenced by the fluctuations in the time patterns of leucine uptake, anaerobic exercise has a more pronounced effect on elevating protein synthesis. Also, the peak response in the different heart fractions are delayed in the anaerobic animals (48 hours) as compared to the aerobic animals (0 and 36 hours). Fluctuations in the time course results also occur in the opposite direction, that is leucine uptake is lower than control levels. It is proposed that these reductions in protein incorporation following exercise are reflective of a true decrement in protein synthesis (Cook et al., 1981). Bostron et al. (1974) speculate that this decrement is due to an increased catabolic effect. The magnitude of the decrement in protein synthesis following exercise is also greater in the anaerobic animals. The hypothesis that protein metabolism is effected by exercise intensity, is supported by the differences in response patterns for leucine incorporation both immediately following and post exercise, between the anaerobic and aerobic groups.

The leucine uptake patterns found in this study suggest that neither exercise stimuli has a selective effect on a single heart fraction. The prominent time course changes occurred in all fractions. These findings are supportive for the theory that exercise does not alter the relative biochemical make up of the heart.

An interesting finding in this study is that the patterns of $^{3}$H-leucine incorporation into the various myocardial fractions within the same exercise group do not follow identical patterns. This indicates that protein synthesis in the heart muscle to some extent may be selective and not regulated in concert. These independent response patterns between different muscle fractions has been shown to occur in skeletal muscle from chronically endurance trained animals (Dohm et al., 1977), and in skeletal muscle from animals exposed to an acute sprint or endurance exercise bout (Wenger et al., 1980).

D. General Discussion

Upon close examination of the data, it is apparent there is considerable intra-group variation in the response to the imposed exercise. It is speculated that a large portion of this variability is due to differences in the relative intensity of both the acclimation training and performance exercise on the animals. The absolute intensity or power output necessary to perform the exercise imposed on the animals in this study can be readily calculated. Because of the difficulty involved in quantifying the relative intensity of effort for an exercising rodent, it is not known what relative stress is incurred by individual animals. That is, what proportion of an individual animal's maximal capacity to perform work is being utilized to carry out the acclimation training and the performance task? From intuitive evaluation of the animal's performance during the course of this experiment it was evident that some animals

were able to complete the daily exercise routine with less difficulty than others. Thus within any group of animals performing the same exercise regime, large differences in the relative stress may exist. Seeing that "intensity" is considered a key factor in producing a training adaptation, the differences in relative intensity or stress may be an explanation for the intra-group variability in the patterns of $^{3}$H-leucine incorporation found in this study. The importance of the intensity of exercise, and its effect on protein metabolism is clearly demonstrated by the differences in the patterns of protein uptake following anaerobic and aerobic exercise found in this study. An alternative explanation for the intra-group variability is put forth by Wenger et al., (1980). They speculate that the protein uptake patterns following exercise are similar between animals but the time course over which they appear may vary. That is, the time when the exercise induced alteration in the muscles biochemical state (as reflected by leucine incorporation) occurs, may not be synchronous in all animals. It is apparent that due to individual physiological differences between animals it is unrealistic to expect an absolute exercise stimuli to have the same effect on protein metabolism in all animals.

Compensatory hypertrophy of the heart is an adaptive response to stress. The characteristics of this hypertrophy, whether it is physiological or pathological are dependent upon the nature of the stress incurred (Dowell et al., 1976). The increased energy demands of performing exercise,

place a volume overload on the heart. This has been found to produce a physiological hypertrophy, that is, the heart weight relative to body weight has been shown to increase (Oscai et al., 1971; Gusti et al., 1978). As well, the heart from an exercise trained animal has been shown to have acquired an improved ability to withstand myocardial pressure overload (Dowell et al., 1976). When considering the effects of physical training on the heart, the mode of exercise, e.g. swimming or running, becomes an important factor. Both modes of exercise are known to have beneficial but different effects on the contractile performance of the heart (Dowell et al., 1976; Schaible et al., 1979). Studies on effects of swimming have attributed the increase in contractility to be a result of enhanced myofibrillar ATPase activity (Wilkerson et al., 1971; Bhan et al., 1975), and an increased capacity of the sarcoplasmic reticulum for calcium uptake (Penpargkul et al., 1977). Similar adaptations have not been demonstrated by endurance running (Baldwin et al., 1977; Penpargkul et al., 1980). It appears that the biochemical adaptations underlying improved heart function resulting from swimming and running differ. This contrast may be due to inherent differences in relative intensity between the two forms of exercise. Myofibrillar ATPase changes have been observed during the early stages of a running program. A study of time course of adaptation (Baldwin et al., 1977) showed significant increase in heart

ATPase activity after four weeks of endurance running. These changes were not evident after six and nine weeks of running. They explained that the initial increase in contractility is due to a greater stress on the cardiovascular system, and that these changes were transitory in nature, regressing as the oxidative capacity of skeletal muscle becomes elevated. Another possible explanation could be that at the onset of training the relative intensity or overload was great enough to produce an adaptation. However, through the remainder of the endurance running program, the intensity may not have been increased sufficiently to produce the degree of overload required either to maintain, or to further elevate the initial increases in contractility. Utilizing a more intense interval training program Baldwin et al., (1977), found that the myofibrillar ATPase activity of the heart remained significantly elevated after nine weeks of training.

Most previous studies examining the effects of an endurance training program on the capacity of cardiac tissue for oxidative energy production, found little evidence of significant changes (Baldwin et al., 1975; Oscai et al., 1971). Baldwin et al. (1977) in their time course study found similar results with endurance training. However, their higher intensity sprint training program did produce increases in the heart's oxidative capacity after six weeks of exercise. These changes in the hearts oxidative capacity appear to parallel Baldwin's previously

mentioned findings regarding the alteration of contractility produced by endurance training. That is, the resultant change in oxidative capacity found after six weeks were no longer evident after nine weeks of sprint training.

Baldwin et al's. results (1977) underscore the importance of intensity of the exercise stress in eliciting a training adaptation. This principle is clearly evident in the present study by the more pronounced effect on protein labelling exhibited in the animals that performed the higher intensity anaerobic exercise. This finding is consistent for all heart fractions measured. A similar response, that is higher levels of protein synthesis in animals subjected to anaerobic exercise as compared to aerobic exercise, has been observed in skeletal muscle (Dallaire 1980).

In response to the purposes of this study as outlined in the introduction it can be concluded that:

- The effect of a chronic anaerobic or aerobic exercise (the exercise acclimation programs) stimulus is to suppress protein metabolism in the separate heart fractions.
- The effect of acute exercise on protein metabolism in the heart appears dependent upon the heart fraction examined, and the nature of the exercise stimuli.
- Anaerobic and aerobic exercise appear to differentially effect the post exercise response patterns for protein uptake. The higher intensity anaerobic exercise seems to have a more pronounced effect on the fluctuations in protein metabolism produced by exercise. As well it

is apparent that the peak elevations for protein labelling in the separate heart fractions are postponed following anaerobic as compared to aerobic exercise.

- The variability in levels of protein uptake evident within the experimental groups, may be indicative of differences in the relative intensity of the imposed exercise stimuli on the individual animals.

III. PRACTICAL IMPLICATIONS

There are several implications that arise from the present study. One is found in the different protein synthesis response patterns between animals within the same group. This indicates that within a group, the same absolute exercise stress illicits a wide range of metabolic response patterns. It is probable that this is, in part, due to differences in the relative stress incurred by the individual animals. This finding underscores the necessity of performance evaluation and individualized exercise perscription. That is, the most effective means of attaining a training adaptation is to design a program in accordance with an individual's work capacities. This ensures that the proper degree of overload or relative stress is being achieved.

A second implication is demonstrated by the different response patterns between the anaerobic and aerobic animals. Because of the differences between species, the finding of this study in terms of time course and magnitude of response would not immediately apply to humans. However, the data

does show that the intensity of the exercise stress is an important factor in governing the metabolic response pattern. The dramatic rise in activity 48 hours post exercise found in the animals exposed to the anaerobic exercise, may infer that longer rest periods are required following this type of exercise than following aerobic exercise. It appears the longer rest interval is necessary to allow the exercise induced alterations in protein metabolism to occur.

Finally, the data indicates that the post exercise response patterns for leucine uptake are not identical for the separate heart fractions. This suggests that the biochemical properties of the heart, represented by the different fractions, to some extent respond independently.

## IV. RECOMMENDATIONS FOR FURTHER STUDY

The discussion in the present study deals with trends evident in the data. To ascertain whether these trends are genuine requires that the intra group variability be reduced. One means of accomplishing this would be to increase the sample size of the experimental groups. The minimum number suggested would be six animals per group. If in fact the amount of biological variability in leucine incorporation does exist, it may be meaningful to examine exercise performance measures and to determine if there are any relationships between these performance measures and leucine incorporation. If such relationships are found, then their effects on the results could be reduced by using blocking procedures in the statistical analysis. Finally, it is

recommended that in future animal research where there is considerable variability in the measured response, that the investigator consider utilizing an inbred strain of animals. A further consideration in this regard is to ensure that the animals are capable of performing the experimental protocol.

It was hypothesized that the intra group variability in the protein uptake, may to some extent result from differences in the relative intensity of the exercise stimuli between animals. To determine if this is a realistic hypothesis requires further research directed at establishing procedures which will enable the relative intensity of effort to be quantified in the exercising rodent. This information would allow the effects of a relative exercise stimulus on protein metabolism to be explored.

The times selected for examing the response in leucine incorporation in the present study were at twelve hour intervals. With this format there were distinct peaks and valleys evident in the measured response. Further delineation to determine where the highest and lowest levels of leucine incorporation occur following exercise requires that the time interval between experimental groups be reduced. It is suggested that following anaerobic exercise the level of leucine incorporation be examined at 4 hour intervals between 24 and 60 hours post exercise. And that following aerobic exercise this rate of sampling should occur between 12 and 48 hours post exercise.

The results in the present study are from animals subjected to a brief acclimation program followed by a

single bout of either endurance or sprint running. Additional research is necessary to determine what the response in heart protein synthesis would be to the many different programs of exercise prescription currently being utilized, and also to determine if the response pattern is similar between trained and untrained animals.

Before the significance of the effects of exercise on protein synthesis in the heart can be realized, further research is clearly required. Hopefully, this research will illustrate the cellular mechanism responsible for the alterations in protein synthesis that result from applying exercises of varying types. Such information will enable one to more accurately monitor and regulate an exercise regimen to insure that the desired results are attained in the most effective and efficient means possible.

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# APPENDIX A

# APPENDIX A

## C. REVIEW OF LITERATURE

Proteins play crucial roles in virtually all biological processes (Stryer, 1975). Because of this importance, the mechanisms comprising protein synthesis have been the focus of an abundance of research. The results of which is our current understanding of the complex processes involved in protein metabolism. These processes include transcription of the required genetic information contained in DNA into mRNA. This reaction requires the enzyme RNA polymerase. The information contained in the mRNA template is translated for the synthesis of proteins. This translation process requires the interplay of tRNA and rRNA. The end result being the sequential addition and binding of amino acids in a specified order to form a new protein molecule (Stryer, 1975). Proteins in a living organism are in a constant state of turnover. The types and amounts of proteins contained in an organ are governed by the balance between the processes of degradation and synthesis.

A muscle's capacity to perform work to a certain extent is dependent on its ability to generate tension. This in turn is dependent upon the quality and quantity of structural or contractile proteins in muscles. Also essential in the capacity of muscles to work is the ability to supply energy to contractile machinery. The energy supply processes are largely contingent on the enzymatic protein levels contained in the muscle. Regular exercise enhances the capacity of the muscle to perform work.

In skeletal muscle, chronic exercise induced adaptations, underlying this enhanced work capacity, are dependent on the nature of the exercise stress incurred. Endurance training has been shown to enhance the oxidative energy producing capabilities of muscles (Holloszy, 1975). Sprint training has been shown to enhance the glycolytic energy producing capabilities (Sjodin, et al., 1976). Elevations in structural protein content have been shown to result from resistance loading (Goldberg, 1975). These exercise induced adaptations result from an alteration in the protein composition of muscle. Such alterations involve a change in the relationship between protein degradation and synthesis. Exactly how exercise affects protein metabolism is not clearly understood at this time. McManus et al. (1975) have shown an elevation in the rate of animo acid incorporating as a result of endurance training. Conversely Dohm et al. (1977) demonstrated either no change or depressed amino acid uptake following an exercise program comprised of both continuous and interval training. Bostrom et al. (1974) demonstrated that the activity of various enzymes involved in energy production varies at different times following an exhaustive exercise bout and that the response pattern is dependent upon the intensity of exercise. Wenger et al. (1980) substantiated Bostrom's results demonstrating that the pattern of amino acid uptake following an acute exercise bout is dependent upon the intensity of exercise and the time of investigation.

Heart muscle is also capable of undergoing adaptation. The adaptation occurs in response to an increased physiological demand or work load applied to the organ. There are a variety of circumstances by which the demands on the heart muscle are elevated. One such example of this is growth. During periods of growth the balance between protein degradation and synthesis is shifted in such a fashion that cardiac size is increased. In embryonic development this heart growth is occurring primarily through mitosis (Zak, 1973), resulting in an increased cell number or hyperplasia. The rodent loses mitotic activity in the cardiac muscle fibers shortly after birth (Zak, 1973). Increases in muscle mass after this early developmental period are brought about primarily by hypertrophy or an increase in myocardial cell size (Zak, 1973). Excluding the normal increase in muscle size due to the growth process, cardiac tissue is capable of undergoing substantial increases in size due to increased work load (Laks et al., 1974). The nature of the increased work load that the heart is subjected to dictates whether the resultant hypertrophy will be either pathological or physiological, i.e., whether heart function is depressed or augmented (Coffelt et al., 1979). Isotonic exercise places a volume overload on the heart. This form of stress (increased work load) enhances heart function (Schaible et al., 1979; Dowell et al., 1976; Carey et al., 1976).

The results from studies investigating the biochemical adaptations in cardiac tissue resulting from an exercise

program are somewhat controversial. Wilkerson et al. (1971) demonstrated that an endurance swimming program resulted in an increased contractility of the heart. This increased contractility is attributted to elevation in myosin ATPase activity. Similar results have been reported by Giusti et al. (1978); Malhotra et al. (1976 and Bhan et al. (1972 and 1978). Studies examining the effects of endurance running on myosin ATPase activity have found that this mode of exercise has little effect on this parameter, (Baldwin et al., 1975; Watras et al., 1979). It appears that there is an inherent difference between swimming and running with regard to the nature of the stress placed upon the heart. Oscai et al. (1971 a & b) have shown that there is no change in the relative respiratory capacity of heart tissue in response to either swimming or running endurance exercise programs. Both modes of exercise have been shown to result in an increased heart to body weight ratio (Oscai et al., 1971; Baldwin et al., 1977; Dowell et al., 1976). From the aforementioned studies, it is apparent that the heart muscle, unlike skeletal muscle, is capable of handling the metabolic and contractile demands of an endurance running program without any relative alteration in the biochemical makeup of the tissue. Also that the most pronounced exercise induced adaptation associated with enhanced heart function appears to be the increase in heart to body weight ratio (Oscai et al., 1971). A possible explanation as to why endurance running has not produced qualitative changes in cardiac muscle may be related to the intensity of

the exercise stress. This possibility is indicated in the data of Baldwin et al. (1977). Their work demonstrated that the higher stress placed upon the heart by interval running resulted in significant increases in myosin ATPase activity. Further to this, their endurance running program did elevate ATPase activity during the early stages of the program (4 weeks) but this change was not evident after 6 and 10 weeks of training. Similar results were found with an oxidative marker enzyme (citrate synthase). That is the interval running program produced significant increases in the activity of this enzyme after 6 weeks of training. However, these differences were not present after 9 weeks of training. The results from Baldwin's group (1977) clearly demonstrate the importance of intensity in eliciting a training adaptation. It is possible that in Baldwin's work the intensity of the training program was not sufficient to maintain or further enhance the changes in oxidative and contractile activity observed in earlier stages of their endurance and interval running programs.

Regardless of the nature of response to exercise, whether it is qualitative or quantitative, changes in the tissue are the product of alterations in protein metabolism. The training adaptation is the cummulative result of repeated acute exposure to exercise. Very few studies have examined how the relationship between protein synthesis and degradation in heart tissue is effected by exercise.

The differences in mechanisms responsible for physiological and pathological hypertrophy in isolated heart

preparations were examined by Hjalmarson et al. (1972). They evaluated the effects of two different workloads, a volume overload and a pressure overload, on protein synthesis activity. The rate of $^{3}H$ phenylaline incorporation was used as an indicator of protein synthesis. The increased preload or volume load was created by increasing the left atrial filling pressure. The increased pressure overload was created by increasing the resistance of the outflow from the left ventricle. It was found that protein synthesis was stimulated in the pressure overloaded hearts. The hearts subjected to the increased volume load did not demonstrate an increase in protein synthesis activity. They concluded that the stimulus responsible for the increase in protein synthesis in the pressure overloaded hearts was the constant increase in muscle fiber length. This persistent increase in fiber length was attributed to the increased tension required during systole and/or the increase in end systolic volume in the pressure overloaded hearts.

Differences between physiological and pathological hypertrophy on heart protein metabolism in vivo were investigated by Dowell et al. (1976). The effects on protein metabolism were inferred by biochemical and fiber size measurements. Physiological hypertrophy was produced by endurance running. Two kinds of simulated pathological hypertrophy were used, moderate pressure overload (DOAC and salt loading), and severe pressure overload (aertic constriction). The animals subjected to the mild

pressure overload demonstrated a 10% increase in fiber growth. There was no indication of cell proliferation in this group. The animals subjected to severe pressure overload demonstrated a significant (35%) increase in muscle fiber size. This group also demonstrated cell proliferation. This proliferation was presumed to occur in non muscle tissue. The endurance running program did not increase left ventricle mass above control levels. However, it did maintain heart size inspite of the exercise induced reduction in body size. The end result was an increase in relative heart size. Because there was no absolute change in heart size, they concluded that in phsiological hypertrophy, in contrast to pathological hypertrophy, there is no increase in protein synthesis activity. It was felt that the effect of exercise was to maintain normal rates of protein metabolism in the heart.

The response of various intra and extra mitochoncrial enzymes activities and RNA content was measured immediately following, and at various time intervals after an acute swimming bout was investigated by Bostron et al. (1974). They found that there was an increase in enzyme activities immediately following the exercise. The activities remained elevated for up to three days post exercise returning to normal after five days. The increases in enzymatic activities were not linear with time nor did they all follow the same response pattern. The RNA content was found to decrease immediately following exercise. It recovered rapidly

and the RNA content was significantly greater than central values one day post exercise, and returned to normal levels after three days of recovery. These results illustrate the significance of the time interval between the final exercise stress and analysis, in studying the effects of exercise on enzyme activity and protein metabolism.

Zimmer et al. (1973) examined the effects of acute exercise on protein synthesis in untrained rats. Animals were sacrificed immediately following and at 1, 2, 3, and 4 hours after sixty minutes of continuous swimming. The effect on protein synthesis was evaluated by the rate of leucine incorporation into the total protein fraction. It was found that there was significant reduction in leucine incorporation immediately following and one hour post exercise. The incorporation rates approached normal levels after two and four hours of recovery. They suggested that the reduced rate of protein synthesis following exercise may be related to the exercise induced energy deficiency in the tissue.

Dohm et al. (1977) conducted a series of experiments investigating the effects of various intensities of endurance training on heart protein metabolism. The demonstrated that endurance training depresses incorporation of leucine into both the stromal and total protein fractions. Relative heart mass was found to increase inspite of the depressed rate of protein synthesis. The authors concluded that the rate of protein degradation must be depressed to an even greater extent than synthesis to account for this

observation. The time elapsed between the final exercise bout and protein analysis was not reported, therefore, the effects of this are not known.

Finally, Cook et al (1981) utilizing an in vitro technique, examined the effects of a single exhaustive bout of endurance swimming on protein synthesis in heart muscle. Protein synthesis was inferred by the incorporation of $^{3}H$-phenylalnine into the total, mitochondrial and extramitochondrial heart fractions. The incorporation was measured immediately following exercise and at 0.5, 1, 2, 4, and 16 hours after exercise. Immediately following the swim there was a decrease in protein incorporation into the three fractions analyzed. The time required for the protein uptake to return to normal following the exercise induced reduction was one hour in the total fraction and two hours in the mitochondrial and extramitochondrial fractions. They found no evidence that protein synthesis was stimulated during the experimental protocol. The data from their estimates of the intracellular availability of $^{3}H$ - phenylalnine suggest that there was no difference in the amino acid pool at any time during the experimental protocol.

# APPENDIX B

Table VII Example of the calculation of protein specific activity (dpm/mg protein) for animal #1 of the anaerobic 12 hour group.

| FRACTION | PROTEIN OD 260am | $\bar{x}$ | -bl* | pr 1 mg | pr 2 mg | COUNTS | $\bar{x}$ | -bl* | S.A. cpm | S.A. dpm |
|---|---|---|---|---|---|---|---|---|---|---|
| TOT | .192-.194 | .193 | .105 | .2500 | .7500 | 737-749 | 743 | 714 | 952 | 2773 |
| MYO | .348-.350 | .349 | .261 | .6214 | 1.8643 | 1307-1334 | 1321 | 1328 | 712 | 2074 |
| MITO | .394-.351 | .373 | .285 | .6786 | | 387-407 | 397 | 368 | 542 | 1579 |
| SOL | .282-.282 | .282 | .194 | .4619 | | 679-710 | 695 | 666 | 1542 | 4492 |

Biuret Blank = .097-.096 Biuret Factor = 0.042

Counts Blank = 28-35

*(-bl = mean of two previous values minus value of blank)

pr 1 - Amount of Protein in a 0.1 ml aliquot of sample

pr 2 - Amount of Protein in a 0.3 ml aliquot of sample

Table VII Serum counts (dpm measured in a 20ml sample of blood taken from experimental animals (A1.A2 - aliquots).

| GROUP | ANIMAL# | A 1 | A2 | GROUP | ANIMAL# | A 1 | A 2 |
|---|---|---|---|---|---|---|---|
| SED.C. | 1 | 13900 | 14536 | SED.C. | 1 | 13900 | 14536 |
| | 2 | 12265 | 13256 | | 2 | 12265 | 13256 |
| | 3 | 14087 | 14764 | | 3 | 14087 | 14764 |
| AN.ACC. | 1 | 11859 | 12588 | AE.ACC. | 1 | 9744 | 10401 |
| | 2 | 10470 | 10821 | | 2 | 11314 | 11416 |
| | 3 | 13813 | 12347 | | 3 | 12065 | 13380 |
| AN.O | 1 | 11871 | 10490 | AE. O | 1 | 12713 | 13547 |
| | 2 | 13673 | 14187 | | 2 | 11943 | 12966 |
| | 3 | 14007 | 15386 | | 3 | 13000 | 14457 |
| | 4 | 11178 | 12800 | | | | |
| AN. 12 | 1 | 12229 | 12894 | AE. 12 | 1 | 8822 | 9405 |
| | 2 | 11417 | 12153 | | 2 | 13876 | 13143 |
| | 3 | 10120 | 10863 | | 3 | 10884 | 12094 |
| AN.24 | 1 | 14300 | 15385 | AE. 24 | 1 | 11617 | 12382 |
| | 2 | 12065 | 12900 | | 2 | 9876 | 11550 |
| | 3 | 14579 | 16100 | | 3 | 10958 | 11711 |
| | 4 | 13069 | 12869 | | | | |
| AN. 36 | 1 | 12869 | 13527 | AE. 36 | 1 | 10355 | 11079 |
| | 2 | 12424 | 13338 | | 2 | 12931 | 14160 |
| | 3 | 11032 | 11824 | | 3 | 9706 | 9206 |
| AN. 48 | 1 | 13640 | 15057 | AE. 48 | 1 | 10370 | 11122 |
| | 2 | 11142 | 12135 | | 2 | 12400 | 12831 |
| | 3 | 10435 | 11128 | | 3 | 11583 | 13194 |
| | 4 | 16508 | 17867 | | | | |
| AN. 60 | 1 | 11711 | 11188 | AE. 60 | 1 | 12866 | 14357 |
| | 2 | 11811 | 11005 | | 2 | 11422 | 10521 |
| | 3 | 11194 | 12176 | | 3 | 11543 | 12192 |
| AN. 72 | 1 | 11744 | 12756 | AE. 72 | 1 | 8852 | 9586 |
| | 2 | 16546 | 15200 | | 2 | 11375 | 12188 |
| | 3 | 11489 | 12713 | | 3 | 11573 | 12847 |

Table VIII Body Weights (g) of experimental animals at time of sacrifice.

| GROUP | ANIMAL# | BODY WT. | GROUP | ANIMAL # | BODY WT. |
|---|---|---|---|---|---|
| SED.C | 1 | 319 | SED.C. | 1 | 319 |
| | 2 | 315 | | 2 | 315 |
| | 3 | 303 | | 3 | 303 |
| AN.ACC. | 1 | 219 | AE.ACC. | 1 | 232 |
| | 2 | 313 | | 2 | 232 |
| | 3 | 287 | | 3 | 276 |
| AN.O | 1 | 252 | AE. O | 1 | 270 |
| | 2 | 261 | | 2 | 248 |
| | 3 | 269 | | 3 | 258 |
| | 4 | 250 | | | |
| AN. 12 | 1 | 236 | AE. 12 | 1 | 211 |
| | 2 | 290 | | 2 | 249 |
| | 3 | 238 | | 3 | 228 |
| AN. 24 | 1 | 208 | AE. 24 | 1 | 243 |
| | 2 | 258 | | 2 | 235 |
| | 3 | 270 | | 3 | 227 |
| | 4 | 256 | | | |
| AN. 36 | 1 | 259 | AE. 36 | 1 | 238 |
| | 2 | 233 | | 2 | 229 |
| | 3 | 229 | | 3 | 257 |
| AN. 48 | 1 | 247 | AE. 48 | 1 | 243 |
| | 2 | 242 | | 2 | 236 |
| | 3 | 188 | | 3 | 268 |
| | 4 | 252 | | | |
| AN. 60 | 1 | 206 | AE. 60 | 1 | 261 |
| | 2 | 243 | | 2 | 258 |
| | 3 | 237 | | 3 | 215 |
| AN. 72 | 1 | 260 | AE. 72 | 1 | 233 |
| | 2 | 221 | | 2 | 262 |
| | 3 | 216 | | 3 | 222 |

Table IX Heart Weights (mg) for all experimental animals.

| GROUP | ANIMAL# | WEIGHT | GROUP | ANIMAL# | WEIGHT |
|---|---|---|---|---|---|
| AE.ACC. | 1 | 532 | AN.ACC. | 1 | 565 |
| | 2 | 533 | | 2 | 752 |
| | 3 | 545 | | 3 | 666 |
| AE. O | 1 | 629 | AN. O | 1 | 497 |
| | 2 | 589 | | 2 | 555 |
| | 3 | 567 | | 3 | 543 |
| AE. 12 | 1 | 567 | AN. 12 | 1 | 438 |
| | 2 | 624 | | 2 | 724 |
| | 3 | 565 | | 3 | 620 |
| AE. 24 | 1 | 577 | AN. 24 | 1 | 479 |
| | 2 | 697 | | 2 | 560 |
| | 3 | 515 | | 3 | 676 |
| AE. 56 | 1 | 513 | AN. 36 | 1 | 551 |
| | 2 | 539 | | 2 | 573 |
| | 3 | 714 | | 3 | 576 |
| AE. 48 | 1 | 631 | AN. 48 | 1 | 641 |
| | 2 | 523 | | 2 | 577 |
| | 3 | 658 | | 3 | 413 |
| AE. 60 | 1 | 604 | AN. 60 | 1 | 556 |
| | 2 | 562 | | 2 | 592 |
| | 3 | 466 | | 3 | 554 |
| AE. 72 | 1 | 534 | AN. 72 | 1 | 616 |
| | 2 | 586 | | 2 | 590 |
| | 3 | 510 | | 3 | 515 |

# APPENDIX C

Table X Incorporation of the radionuclide L-(4,5 $^{3}H$)-Leucine (dpm/mg protein) in all fractions (total homogenate-TOT, myofibrillar-nuclear-MYO, mitochondrial-MIT and soluble -SOL) of the myocardial muscle from the animals in the sedentary control and endurance groups.

| GROUP | FRACTION | ANIMAL NUMBER 1 | 2 | 3 | MEAN |
|---|---|---|---|---|---|
| SED.C. | TOT | 17344 | 16036 | 12610 | 15330 |
| | MYO | 10152 | 8165 | 7702 | 8673 |
| | MIT | 4163 | 2065 | 3175 | 3134 |
| | SOL | 5246 | 5089 | 3764 | 4700 |
| AE.ACC. | TOT | | | | |
| | MYO | 2427 | 2138 | 2330 | 2298 |
| | MIT | 1847 | 2695 | | 2271 |
| | SOL | 4256 | 3630 | 3437 | 3774 |
| AE.O | TOT | 3222 | 3863 | 2802 | 3296 |
| | MYO | 2537 | 3158 | 2316 | 2670 |
| | MITO | 2470 | 3079 | 2843 | 2797 |
| | SOL | 3982 | 5080 | 3854 | 4305 |
| AE. 12 | TOT | 4801 | 2604 | 3231 | 3545 |
| | MYO | 3656 | 2042 | 2561 | 2753 |
| | MITO | 3376 | 1730 | 2447 | 2518 |
| | SOL | 5197 | 3190 | 3898 | 4095 |
| AE. 24 | TOT | 3781 | 3685 | 3123 | 3530 |
| | MYO | 2566 | 3009 | 2223 | 2599 |
| | MITO | 1993 | 2258 | 1620 | 1957 |
| | SOL | 4559 | 3490 | 1911 | 3320 |
| AE. 36 | TOT | 3656 | 3857 | 1031 | 2848 |
| | MYO | 2910 | 2811 | 886 | 2202 |
| | MITO | | | | |
| | SOL | 5028 | 4597 | 3700 | 4442 |

Table X (continued)...Incorporation of the radionuclide L-(4,5 $^{3}H$)-Leucine (dpm/mg protein) in all fractions (total homogenate-TOT, myofibrillar-nuclear-MYO, mitochondrial-MIT and soluble-SOL) of the myocardial muscle from the animals in the sedentary control and endurance groups.

| GROUP | FRACTION | ANIMAL NUMBER 1 | 2 | 3 | MEAN |
|---|---|---|---|---|---|
| AE. 48 | TOT | 2395 | 3799 | 3650 | 3281 |
| | MYO | 2060 | 2386 | 2569 | 2338 |
| | MITO | 2269 | 1541 | 2645 | 2152 |
| | SOL | 2808 | 3266 | 4110 | 3395 |
| AE. 60 | TOT | 2791 | 2537 | 3300 | 2876 |
| | MYO | 2360 | 2048 | 2479 | 2295 |
| | MITO | 1028 | 1669 | 2298 | 1665 |
| | SOL | 3362 | 3469 | 4099 | 3643 |
| AE. 72 | TOT | 2869 | 2412 | 2447 | 2576 |
| | MYO | 2406 | 1827 | 1949 | 2061 |
| | MITO | 1789 | 2121 | 1754 | 1888 |
| | SOL | 3697 | 3041 | 3199 | 3312 |

Table XI Incorporation of the radionuclide L-(4,5 $^{3}H$)- leucine (dpm/mg protein) in all fractions (total homogenate-TOT, myofibrillar-nuclear-MYO, mitochondrial-MIT and soluble-SOL) of the myocardial muscle from the animals in the sedentary control and sprint groups.

| GROUP | FRACTION | ANIMAL NUMBER 1 | 2 | 3 | MEAN |
|---|---|---|---|---|---|
| SED.C. | TOT | 17344 | 16036 | 12610 | 15330 |
| | MYO | 10152 | 8165 | 7702 | 8673 |
| | MITO | 4163 | 2065 | 3175 | 3134 |
| | SOL | 5246 | 5089 | 3764 | 4700 |
| AN.ACC. | TOT | 3047 | 4346 | 1998 | 3130 |
| | MYO | 2348 | 3295 | 1506 | 2383 |
| | MITO | 2124 | 3525 | 1145 | 2265 |
| | SOL | 4480 | 5637 | 2756 | 4291 |
| AN. 0 | TOT | 3662 | 3102 | 3152 | 3305 |
| | MYO | 2537 | 3158 | 2316 | 2670 |
| | MITO | 2759 | 1972 | 1963 | 2231 |
| | SOL | 4536 | 4236 | 3854 | 4209 |
| AN. 12 | TOT | 2773 | 3245 | 3266 | 3095 |
| | MYO | 2074 | 2779 | 2540 | 2464 |
| | MITO | 1579 | 2144 | 1888 | 1870 |
| | SOL | 4492 | 4576 | 4445 | 4504 |
| AN. 24 | TOT | 3592 | 2095 | 2304 | 2664 |
| | MYO | 2767 | 1442 | 1658 | 1956 |
| | MITO | 3012 | 1095 | 1576 | 1894 |
| | SOL | 5080 | 3065 | 3172 | 3772 |
| AN. 36 | TOT | 2462 | 2217 | 2566 | 2415 |
| | MYO | 2179 | 1550 | 1949 | 1893 |
| | MITO | 1119 | 1267 | 953 | 1113 |
| | SOL | 3070 | 3297 | 3158 | 3108 |
| AN. 48 | TOT | 4253 | 4716 | 4640 | 4536 |
| | MYO | 2840 | 3113 | 3166 | 3039 |
| | MITO | 2654 | 3909 | 2293 | 2952 |
| | SOL | 4949 | 4923 | 4996 | 4956 |

Table XI (continued)...Incorporation of the radionuclide L-(4,5 $^{3}H$)-leucine (dpm/mg protein) in all fractions (total homogenate-TOT, myofibrillar-nuclear-MYO, mitochondrial-MIT and soluble-SOL) of the myocardial muscle from the animals in the sedentary control and sprint groups.

| GROUP | FRACTION | ANIMAL NUMBER 1 | 2 | 3 | MEAN |
|---|---|---|---|---|---|
| AN. 60 | TOT | 3353 | 3190 | 3519 | 3354 |
| | MYO | 2185 | 2057 | 2450 | 2231 |
| | MITO | 1486 | 1299 | 1626 | 1470 |
| | SOL | 4603 | 4058 | 4218 | 4293 |
| AN. 72 | TOT | 3694 | 2563 | 3027 | 3095 |
| | MYO | 3012 | 1841 | 1911 | 2255 |
| | MITO | 2217 | 1660 | 1305 | 1727 |
| | SOL | 3539 | 2715 | 3860 | 3371 |

# APPENDIX D

Table XII Calculation of the conversion factor used to change cpm to dpm. An internal standard of known radioactivity was used. Data from animal 1 (AE.60) was used for standardization.

| FRACTION | MUSCLE | ORIGINAL cpm | NEW cpm | DIFFERENCE |
|---|---|---|---|---|
| TOT | H | 590 | 37950 | 37360 |
| | | 600 | 37567 | 36967 |
| | S | 390 | 36817 | 36427 |
| | | 423 | 37177 | 36694 |
| | P | 433 | 36100 | 35667 |
| | | 424 | 39533 | 39109 |
| MYO | H | 843 | 37467 | 36624 |
| | | 841 | 38500 | 37659 |
| | S | 628 | 37817 | 37189 |
| | | 648 | 36717 | 36069 |
| | P | 585 | 38250 | 37765 |
| | | 600 | 38750 | 38150 |
| MIT | H | 264 | 39783 | 39519 |
| | | 260 | 38950 | 38690 |
| | S | 129 | 37917 | 37788 |
| | | 140 | 39383 | 39243 |
| | P | 124 | 39417 | 39293 |
| | | 126 | 39367 | 39241 |
| SOL | H | 595 | 39467 | 38872 |
| | | 602 | 38867 | 38265 |
| | S | 255 | 40160 | 39905 |
| | | 259 | 38217 | 37958 |
| | P | 329 | 39983 | 39654 |
| | | 304 | 38617 | 38313 |
| SERUM | | 14914 | 51700 | 36786 |
| | | 15279 | 58125 | 42846 |
| BLANK | | 27 | 38517 | 38490 |
| | | 28 | 37200 | 37172 |
| | | | Mean = | 38133 |

Internal Standard = $1.327 \times 10^6$ dpm/gm solution
100 ul=0.0837 gm solution
Radioactivity added to samples = 111,070 dpm/100 ul
Conversion factor for cpm to dpm on Beckman LS 250 counter: 111,070 / 38,133 = 2.913

* The data from the skeletal muscles Soleus-S, and Plantaris-P, used with the permission of Dr. J. Dallaire.

# BIURET TECHNIQUE*

## Reagent Preparation

1.5g of $CuSO_4.5H_2O$ and 6.0g of $NaKC_4H_4O_6.4H_2O$ were mixed in approximately 500ml of distilled water.

300ml of 10% NaOH was added to the above solution with constant swirling.

The solution was then diluted to 1.0l with distilled water and stored in a 'Teflon' bottle.

## Procedure

0.5ml of the above reagent was added to 0.1ml of sample. The mixture was agitated and left to stand for 10 min. at room temperature.

The Optical Density was spectrophotometrically measured at a wavelength of 540nm and the value multiplied by the standard curve factor (0.042) established for the Biuret reagent. The results were expressed as mg of protein in 0.01ml of sample.

* Modified from Gornall, A.G., et al., Journal of Biological Chemistry 177:751-66, 1949.

Table XIII Sacrifice time schedule for experimental animals.

| GROUP | ANIMAL # | TIME OF SACRIFICE(HRS) | GROUP | ANIMAL # | TIMEOF SACRIFICE(HRS) |
|---|---|---|---|---|---|
| SED.C. | 1 | 8:30 | | | |
| SED.C. | 2 | 11:30 | | | |
| SED.C. | 3 | 14:30 | | | |
| AN.ACC. | 1 | 14:00 | AE.ACC. | 1 | 16:30 |
| AN.ACC. | 2 | 8:00 | AE.ACC. | 2 | 10:30 |
| AN.ACC. | 3 | 7:30 | AE.ACC. | 3 | 7:30 |
| AN. O | 1 | 17:30 | AE. O | 1 | 11:00 |
| AN. O | 2 | 12:30 | AE. O | 2 | 14:00 |
| AN. O | 3 | 8:30 | AE. O | 3 | 15:30 |
| AN. O | 4 | 13:30 | | | |
| AN. 12 | 1 | 11:30 | AE. 12 | 1 | 7:30 |
| AN. 12 | 2 | 19:30 | AE. 12 | 2 | 19:30 |
| AN. 12 | 3 | 8:30 | AE. 12 | 3 | 12:30 |
| AN. 24 | 1 | 14:30 | AE. 24 | 1 | 10:30 |
| AN. 24 | 2 | 17:30 | AE. 24 | 2 | 13:30 |
| AN. 24 | 3 | 20:30 | AE. 24 | 3 | 16:45 |
| AN. 24 | 4 | 7:30 | | | |
| AN. 36 | 1 | 11:30 | AE. 36 | 1 | 7:30 |
| AN. 36 | 2 | 23.30 | AE. 36 | 2 | 19:30 |
| AN. 36 | 3 | 19:30 | AE. 36 | 3 | 21:30 |
| AN. 48 | 1 | 11:00 | AE. 48 | 1 | 10:30 |
| AN. 48 | 2 | 17:30 | AE. 48 | 2 | 13:30 |
| AN. 48 | 3 | 20:30 | AE. 48 | 3 | 16:30 |
| AN. 48 | 4 | 17:00 | | | |
| AN. 60 | 1 | 11:30 | AE. 60 | 1 | 19:30 |
| AN. 60 | 2 | 23:30 | AE. 60 | 2 | 19:30 |
| AN. 60 | 3 | 8:30 | AE. 60 | 3 | 21:30 |
| AN. 72 | 1 | 14:30 | AE. 72 | 1 | 10:30 |
| AN. 72 | 2 | 17:30 | AE. 72 | 2 | 13:30 |
| AN. 72 | 3 | 20:30 | AE. 72 | 3 | 16:30 |

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